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I — INTRODUCTION

The following microbiological study, limited to the cultural and biochemical characters, represents only part of a complete study that we are making on Malassez and Vignal's bacillus and its role in human and animal pathology.

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After having for a long time occupied only the attention of veterinarians, Malassez and Vignal's bacillus has been creating in the last few years a new interest by reason of the extension of our knowledge of its role in human pathology. This knowledge, originally limited to a few rare observations of a septicemic form (less than twenty in the world-wide bibliography) and to a few still more rare observations of localized forms (ocular, pulmonary and perhaps splenic), has been enriched by the discovery of a major form: acute mesenteric adenitis with Malassez and Vignal's bacillus (Knapp and Masshoff, 1954; Knapp, 1954; Graber and Knapp, 1955; G. Girard, 1954-1959). This organism has now become almost commonplace and several hundred cases of it have been diagnosed in less than ten years throughout the world, more than one hundred of which in France during the last four years.

In a second stage, the individualization of purely digestive forms (Kulbmann and Hermann, 1955; Braun and Muller, 1957; Knapp, 1959; J. Schmidt, 1959; Mollaret, 1960-1962), of forms limited to a nodose erythema (Morger, 1962, Mollaret, 1962) or of ganglionic forms with a localization that is no longer mesenteric but cervical, (Mollaret, 1962) shows the increasingly important position that this germ is occupying from now on, both in medical and surgical pathology.

In another field, we seem to be witnessing at present an extension of the geographic distribution of the infection among animals, and this role, increasing as it becomes better known, of Malassez and Vignal's bacillus both in human and in veterinarian medicine justifies the bacteriological study that we present here.

Finally, from the doctrinal point of view, the study of this germ is particularly interesting in view of its relationships with the plague bacillus, relationships that are close enough to raise a double problem, one purely practical, the problem of criteria of differentiation of the two germs, the other dogmatic and taxonomic, concerning their exact degree of relationship and their position in systematology.

Since we had a collection of strains of Malassez and Vignal's bacillus that we received from Truche, Dujardin-Beaumetz, G. Girard, and increased from time to time with strains that we isolated in man and animal, in addition to those that were sent to us for identification and typing [See Note], and numerous strains of Yersin's bacillus kept in the Plague Department of the Pasteur Institute and in its branches outside of the capital, we tried to determine the present state of knowledge on the cultural and biochemical character of Malassez and Vignal's bacillus.

([Note:] We are particularly grateful to Dr. A. Lucas, Director of the Central Laboratory for Veterinary Research of the Ministry of Agriculture, for the abundant material for which we are

indebted to him.)

Our study was extended to 617 strains of this germ, 497 of which were isolated in France and 120 abroad [See Note], of the following animal origins:

Hares	286 strains
Guinea-pigs	53
Birds	45
Men	36
Rabbits	37
Monkeys	23
Rats and mice	19
Turkeys	14
Various	104

and that are distributed as follows in the five serologic types known at present:

Type I	402
Type II	119
Type III	27
Type IV	6
Type V	6
Unclassified (auto-agglutinative) strains	57

([Note:] A number of them are due to the kindness of Mme. G. V. Bulanova-Zhuchenko (Gamaleya Institute, Moscow), Mme. L. A. Timofeyeva (Institute of Antiplague Research, Irkutsk), Mr. W. Frederiksen (Government Serum Institute, Copenhagen) and Mr. P. Zwart (Institute for Tropical and Protozoan Research of the Royal University of Utrecht.).)

We have had the privilege, thanks to Dr. H. Marneffe, Assistant Director of the Pasteur Institute at Paris, of being able to undertake our work under Dr. G. Girard, head of the Plague Department of the Pasteur Institute, and to benefit in that way from his invaluable experience.

Another privilege was granted to us by Professor V. van Straelen, President of the Institute of National Parks in the Belgian Congo: the privilege of working in 1955-1956 and 1957-1958 on the exceptional staff of the Albert National Park, ancient central African home of the plague. Our interest in this germ was born while we were isolating Malassez and Vignal's bacillus [See Note] in Dendrohyrax caught in Ruwenzori (1958).

([Note:] Henceforth we shall use only the term Malassez and Vignal's bacillus and reject the terms Pasteurella pseudotuberculosis and "pseudotuberculosis" in view of the constant confusion they create. Malassez and Vignal's bacillus, which was as unfortunately classified in the genus Pasteurella as were the agents of plague and tularemia, does not have, as do the other two, any other connection with Pasteurella septica than a morphological resemblance that a number of other species also have. In daily practice (in particular in requests for serologic examinations), there is constant confusion between Pasteurella septica and Pasteurella pseudotuberculosis, both of which have their own human pathology. The term Pasteurella, therefore, leads to confusion.

The term pseudotuberculosis is just as dangerous: Malassez and Vignal's bacillus, gram-negative and non-acid-alcohol resistant, has no connection, either with the tuberculosis bacillus or with the paratuberculosis bacilli, or with Corynebacterium pseudotuberculosis

(Preisz and Nocard's bacillus), or with the great number of "pseudotuberculoses" whose various agents, inert or alive, bacterial, mycotic, parasitic, etc., are perpetually confused with Pasteurella pseudotuberculosis.

The most constant and serious confusions occur between "tuberculosis", "paratuberculosis" and "pseudotuberculosis". We can no longer count the number of patients subjected to Rimifon after a positive serodiagnosis of "pseudotuberculosis." Therefore, we shall henceforth use only the term Malassez and Vignal's bacillus and we hope to see both the term of Pasteurella and of pseudotuberculosis rejected in so far as it is concerned.)

We express particular gratitude to Madame D. Piechaud and to Mr. M. Piechaud, who discovered the first French case of acute mesenteric adenitis with Malassez and Vignal's bacillus (1952), for the experience from which we derived great profit.

Finally, we are very grateful to Mr. A. Chevalier and to Mademoiselle M. C. Deplanche for the very efficient technical assistance they gave us in this work, and to Mademoiselle S. Lagniez, whose collaboration we owe to the financial generosity of the SPECIA Laboratories.

II — CULTURAL CHARACTERS

1. General culture conditions.

Nutritive requirements.

Malassez and Vignal's bacillus grows easily in the usual mediums and has no special nutritive requirements.

Berkman (1942), while he was studying comparatively the requirements of the different Pasteurella for accessory growth factors, demonstrated that Malassez and Vignal's bacillus, like Yersin's bacillus, develops perfectly in a synthetic medium containing 15 amino acids, mineral salts and glucose (differently from P. septica and P. tularensis), as it does in hydrolysate of gelatin, in which P. septica does not grow without the addition of nicotinamide and pantothenic acid.

These modest nutritive requirements are sufficiently clear, so far as Yersin's bacillus is concerned, to recommend different techniques for differentiating the two germs:

a. Plain gelose. The utilization of simple, plain gelose would permit, according to Bezssonova, a distinction based on the fact that in this medium Yersin's bacillus usually grows restrictedly, in isolated colonies, whereas Malassez and Vignal's bacillus is abundantly developed after 24 hours. Taylor (1933), in the Report of the Haffkine Institute for the Year 1931, recommends the following differentiation method based on seeding a small number of germs on plain gelose: with the aid of a 1 mm rod, dilutions of the two germs are made in tubes containing 10 ml of physiologic serum. These dilutions are then seeded by spreading part on gelose with blood with a rod 1 mm in diameter and part on plain gelose, but with a 5 mm rod. Yersin's bacillus yields distinct colonies on gelose with blood, but no culture appears on plain gelose, whereas Malassez and Vignal's bacillus grows equally well on both mediums.

Dujardin-Beaumetz (1934), like G. Girard, likewise sees in the unequal rapidity of development of the two germs a good differentiation criterium at the time of isolation: "every germ that, when it leaves the organism, grows rapidly on gelose is not a plague bacillus" (G. Girard).

b. Bezssonova's "acid poor gelose". This medium was proposed by Bezssonova (1929) who demonstrated that, although cultures of Malassez and Vignal's bacillus and Yersin's bacillus are identical on gelose without bouillon, or on a culture medium with a low pH, the combination of four factors, "poor" gelose (in which bouillon is replaced by plain or distilled water), a weak gelose concentration (3 parts in 100), low pH (5.9 - 6.1) and a small amount of inoculum, permits the production of a culture only with Malassez and Vignal's bacillus.

c. "Exhausted" mediums and "vaccinated" mediums. According to Fabiani (1933), tubes of gelose that have been used for several Yersin's bacillus cultures in succession and have had the bacilli removed by washing with physiologic serum, become unfit for cultivating this germ, but still permit the development of Malassez and Vignal's bacillus and vice-versa.

Petragnani (1937), recommended, in order to differentiate the plague bacillus "from other germs" (although without specifying Malassez and Vignal's bacillus by name) the use of gelose that has been used for cultivating Yersin's bacillus and then autoclaved and rehydrated. Yersin's bacillus would not grow any more in these "exhausted" mediums, in contrast to the other species.

The use of these two techniques did not yield any conclusive results.

c. Synthetic mediums. Mayeda (1927) proposed a synthetic medium free from proteins, not suited for the growth of Yersin's bacillus or for P. septica, but in which Malassez and Vignal's bacillus develops in three days.

The addition of glycerin (Baumann, 1927; Poppe 1928; Dujardin-Beaumetz, 1934) or of sugar (Poppe) or of serum (Baumann, 1927) favored the cultures. Cultures were especially abundant in Nicolle and Alilaire's medium (gelose with glycerin peptone bouillon (1 part in 100) and macerated potato, according to Dujardin-Beaumetz (1934) and Hauduroy (1953). According to Topley and Wilson (1954) and Knapp (1960) and ourselves, the cultures are not influenced by the addition of serum or of glucose and they are slightly inhibited by glycerin.

According to Jackson and Morris (1961), Malassez and Vignal's bacillus, as well as Yersin's bacillus, does not develop in human serum beyond 24 hours, unless iron is added to it.

Culture temperature.

The extreme culture temperatures compatible with the vitality of the germ are 4° and 43° for Wilson and Miles (1955) and Bergey (1957), 0° to 40°-41° for Zlatogoroff (1904), 0° to 45° for Toumanski and colleagues (1935).

Zagari (1890) gives the figures of 46° to 48° as the upper culture limit, whereas Zlatogoroff (1904) and Dujardin-Beaumetz (1934) state that there is no more growth above 40°-41°. Lucet (1898), nevertheless, obtained cultures at 40°-41° and Toumanski and colleagues (1935), who observed growth between 40° and 45°, give this last figure as the temperature limit for the development of Malassez and Vignal's bacillus and Yersin's bacillus.

The lower temperature limit was probably +5°, according to Poppe (1928). For Dujardin-Beaumetz, colonies do not appear before the seventh day between +8° and +10°. For Zlatogoroff, the germ would probably not develop at 0°, but Toumanski and colleagues obtained a culture at that temperature and observed that at extreme temperatures, both upper and lower, Malassez and Vignal's bacillus cultures are always more luxuriant than cultures of Yersin's bacillus, a result that contradicts those obtained by Galli-Valerio (1903) who observed, when he was cultivating the two germs comparatively between +1° and -5°, a slight development of Yersin's bacillus commencing with 15th hour and of Malassez and Vignal's bacillus only after 48 hours. According to Sokhey and Habbu (1943), Yersin's bacillus would probably develop in bouillon between -2°C and +45°C.

According to Zlatogoroff, the slowing down of the growth of Malassez and Vignal's bacillus appears below 18°-20°; Knapp (1959) confirmed these last figures: the cultures are slightly retarded at 18°-20°, but after two or three days at this temperature they display the same density as the one to two day cultures at 30° or 37°.

The optimum temperature likewise varies according to the writers: the best yield was obtained between 30° and 38° for Zlatogoroff and for

Poppe, 37° for Lucet as well as for Courmont and Panisset (1914) and for Preston and Maitland (1952), between 22° and 30° for Zagari (1889) who observed a poor yield at 38°-39°, 30° for Thal (1954) and for Wilson and Miles (1955); according to Knapp (1959) the greatest multiplication of the germ takes place at 30° and the optimum is included between 24° and 37°. Personally, we locate this optimum between 28° and 37°, but two points must be taken into consideration:

a. On the one hand, the vitality of the germ is influenced very much by the culture temperature. Although a number of authors were able to keep cultures in the incubator at 37° for varying periods of time (three months on gelose for Lucet (1898), seventy-two days on gelose and thirty-five days in bouillon at 38° for Megnin and Mosny (1891); others observed a rapid stoppage of the cultures. Thus Gate and Billa (1928) ascertained that the germ did not develop at 37° beyond two to three generations, a figure that is certainly quite insufficient, but we also at times saw cultures die off beyond one week at 37°.

On the contrary, their vitality is altered less at 28°, as had been demonstrated by Lucet (1898) whose strains were still transplantable after four months of cultivation at 28°, whereas they were not transplantable after three months at 37°.

b. On the other hand, the culture temperature influences a number of characters of the germ, such as mobility, dissociation, virulence and various biochemical qualities. This influence will be discussed in detail in the respective chapters; let us only say here that the tendency to form rough variants and the tendency to lose mobility, virulence and even vitality at 37° (although germs in phase S retain their maximum virulence between 22° and 28° -- P. Boquet, 1937, Preston and Maitland, 1952), should make us prefer systematically culture temperatures included between 20° and 30°. Nevertheless, certain tests require a particular thermic optimum: 37° for examining urease, 18° for a culture in Simmons' medium, 28° for attacking sodium malonate (Mollaret, 1961).

It appears, moreover, that the incubation temperature may influence the culture at the time of isolation: Rosenwald and Dickinson (1944), Fey (1956) and Knapp (1956) discovered some human or animal strains that grew, when isolated, only at room temperature. This is the temperature recommended by Pfeiffer as far back as 1889.

pH:

The optimum pH required by Malassez and Vignal's bacillus is placed between 6 and 8 (Knapp, 1959). The extreme limits of tolerance were 5.8 and 8.4 for E. Korobkova (1929), 6.5 and 9 for J. Dumas (1951), 5.2 and 10 for Knapp.

According to Bessonova (1929), although Malassez and Vignal's bacillus and Yersin's bacillus tolerate the same acidity (5.9 to 7) in the usual gelose mediums, all the strains of Yersin's bacillus did not develop in geloses at pH 6.4 and below without a definite concentration of gelose (2 parts in 100), as contrasted with Malassez and Vignal's bacillus that always grows on this medium (Bessonova's acid poor gelose).

The acidity of the mediums involves the appearance of long, filamentous forms, like the ones that Gate and Billa (1928) observed on Sabouraud's medium. It also favors the occurrence of R-variants (Knapp, 1959) and tends to interrupt the mobility of the germ.

Although, according to Preston and Maitland, pH variations have no influence on mobility, according to P. Boquet, who checked paral-

lelly variations in pH and mobility at 28° in a Sauton synthetic culture medium containing 2 parts in 1,000 of glucose in place of glycerin, mobility decreases as acidity increases. According to Knapp, a few rare strains may still retain mobility at pH 10, and all are immobile at pH 4. The optimum pH for obtaining a good mobility is between 7 and 7.3 (P. Boquet, 1937, Preston and Maitland, 1952).

Influence of atmospheric oxygen.

The bibliography on the subject has been especially rich in differences in opinion on this point ever since Nocard and Masselin (1889) established the potential aerobic and anaerobic character of Malassez and Vignal's bacillus.

The possibility of development in anaerobiosis was subsequently denied by Zlatogoroff (1904), Megnin and Mosny (1891), who state that "repeated attempts to cultivate in a vacuum or in the presence of an inert gas, such as illuminating gas, have consistently remained without result", by Messerschmidt and Keller (1914), Kakehi (1916) whose cultures "on gelose in a vacuum in accordance with Bulloch's method (pyrogallol, potash, washin with hydrogen) did not show any appreciable growth in three d- out spread rapidly in the presence of oxygen", by Schutze (192?) for whom "strict anaerobiosis inhibits growth completely" and by Dujardin-Beaumetz (1934) whose cultures "were almost zero when deprived of air". According to Sacerdotti (1905), "anaerobic cultures on both gelatin and gelose by exception give rise to growth. If, after eight to ten days of anaerobiosis, the cultures are again put in contact with oxygen, the development is irregular. It is regular only if the germ has remained not over two to three days in a medium deprived of oxygen". Wilson and Miles (1955), finally, did not obtain any culture in stab-gelose.

Other writers are of a less definite opinion: for example, Lucet (1898) for whom "the cultures are less abundant in a vacuum than in air", or Courmont and Panisset (1914), Barsini (1935) and Winkle (1955) who observed a better growth in the presence of oxygen.

On the other hand, the potential aerobic-anaerobic character is affirmed by Preisz (1894), who states, however, that "the bacillus grows deep in the puncture and in the gelatin layer, even though it is very thin, much more poorly than on the surface", by Pfeiffer (1899), Nocard and Masselin (1899), by Cipollina (1900) who considers the germ potentially anaerobic, although it "seems to grow less well in the absence of oxygen", by Vicenzi (1909), Poppe (1913), Saisawa (1913), Baumann (1927), Weitzenberg (1934 and 1935), Bergey (1957), etc.

Knapp (1959) confirmed the potential aero-anaerobic character of Malassez and Vignal's bacillus with the following reservations that explain the differences in opinion of the foregoing writers: on the one hand, the inhibitive action of anaerobiosis on growth is a function of the medium used. Thus, whereas growth at 37°, 22° and 2° in tryptose bouillon is identical in aerobiosis, it is slowed down by anaerobiosis in plain bouillon or in bouillon with serum or glucose added. On the other hand, the behavior in anaerobiosis may vary with the strains and with their age. That is why some of them, when they leave the organism, are incapable of developing in anaerobiosis at 37° and are cultivated only in anaerobiosis at 22° (Knapp, 1960). Finally, the tendency toward dissociation and the appearance of R-forms is greater in anaerobiosis (Knapp, 1959).

Although Schütze and Hassanein (1929) demonstrated that, in

liquid culture mediums, oxygen exerted an inhibitive action on Yersin's bacillus and not on Malassez and Vignal's bacillus, and although they proposed using this character to distinguish between the two germs, the differences in behavior of Malassez and Vignal's bacillus and Yersin's bacillus in deaerated solid mediums (deep gelose, VF glucose gelose, Legroux's gelatin gelose) do not seem to have received any attention from a practical point of view, in spite of the consistency of the aspects displayed. We seeded, by way of comparison, several hundred strains of both germs in these different mediums and we always obtained a development of Malassez and Vignal's bacillus in the entire upper part of the tubes [See Note 1], whereas the plague bacillus develops only in the anaerobiosis area and only rarely yields colonies in the aerobic area of the mediums [See Note 2], characters already observed by M. Piechaud. Therefore, Malassez and Vignal's bacillus must be considered a potentially aerobic-anaerobic, while the behavior of Yersin's bacillus in deep gelose tends to confirm the results of Rao (1940) and Herbert (1949). According to Girard and Neel (1946), there probably is reason, moreover, in so far as the latter germ is concerned, to consider the number of germs seeded and the date that colonies appear: "Let us note," these authors write, "that, although with seeding on the order of 500,000 to one million germs colonies are observed in the entire upper part of deep glucose gelose, with the amounts of germs that we used in this experiment (varying from 50 to 50,000), during the first two to three days we saw colonies only in the anaerobiosis area of the gelose; the development was seen in the mesoaerobic and aerobic areas only after this period of time. We observed the same phenomenon with VF glucose deep gelose, 2 parts to 1,000, that yields results comparable to the ones obtained with Uclaf glucose peptone stab gelose."

([Note 1:] According to J. Dumas (1958) and Percebois (1961), the colonies were probably more numerous in the area of aerobiosis; we did not verify this.)

([Note 2:] Except for Girard and Robic's E.V. strain that grows in the entire upper part of the medium.)

Culture based on small seedings.

The facility with which Malassez and Vignal's bacillus is cultivated is contrasted with the difficulty, even the impossibility, of obtaining a development of Yersin's bacillus in bouillon or on gelose when using a small number of germs, a difficulty that numerous writers have pointed out and attempted to explain as due to various causes: presence of inhibitive substances (Drennan and Teague, 1917; Girard, 1956), oxidation of the culture medium (Wright, 1934), production of oxygenated water (Rao, 1939; Herbert, 1949), etc.

Schütze and Hassanein (1929) confirmed the ease of development of Malassez and Vignal's bacillus, even using a reduced inoculum, when they attributed a special sensitivity of Yersin's bacillus to atmospheric oxygen, and when they made comparative seedings of Malassez and Vignal's bacillus, Yersin's bacillus and P. septica with a small number of germs and by adding sodium sulfite or blood as a reducing agent of the medium.

In a more general way and in comparison with Yersin's bacillus, in the same mediums and under the same conditions, Malassez and Vignal's bacillus is capable of being cultivated using a small number of germs. Seeding of sufficiently developed dilutions of both germs on

gelose shows the much greater facility of Malassez and Vignal's bacillus for growing on the basis of a light seeding. The same is true when pathologic products, infinitely less rich in bacilli, are used when it is a question of Malassez and Vignal's bacillus than when it is a question of a plague bacillus. Bezsonova, Egorov and Melnikova (1940) proposed differentiating these two germs by seeding them on gelose using progressively decreasing dilutions.

Girard (1956) demonstrated that the inhibitive action of beef bouillon on the growth of Yersin's bacillus, a phenomenon whose practical consequences Baltazard and Bahmanyar (1960) appear to be the only ones to have emphasized, was without effect with regard to Malassez and Vignal's bacillus: "Quite some time ago E. Dujardin-Beaumetz had told us that it was very difficult to obtain well isolated colonies of P. pestis on gelose; either nothing grew if the seeding was too small, or the colonies were confluent with the denser inoculates. In the plague laboratory solid or liquid mediums were prepared with macerated beef with the addition of an equal volume of Martin's peptone or of 20 g per liter of a commercial peptone. On the other hand, nutrient gelatin was completely satisfactory from this point of view, although it differed from the preceding mediums only by the substitution of gelatin for gelose. R. Devignat (in a personal communication) has always used gelatin successfully to count the viable germs in a given suspension of P. pestis. Therefore, we wondered if gelatin did not have, like blood or serum, the power to neutralize the inhibitory substances in bouillon. The experiment confirmed this. We prepared a nutrient gelatin according to the formula for our medium A (nutrient gelose with bouillon plus 20 parts in 1,000 of PTV peptone) in which the gelose was replaced with 150 parts in 1,000 of gelatin. One drop of P. pestis dilution, that was incapable of growing on the original medium A, in a Petri dish, produced, after spreading out, the development of colonies in a number approximately equal to the number provided by the peptone gelose. Only a slight delay was noted in the appearance of colonies on gelatin, although the dishes had been left, in both cases, at laboratory temperature, about 20°C.

Let us add that the addition of blood to the peptone gelose does not introduce any favorable element in this medium that is self-sufficient. However, it is not detrimental to it either.

It was interesting to determine if the inhibitory effect of beef bouillon was also effective on Pasteurella pseudotuberculosis by virtue of affinities between this micro-organism and P. pestis. It was not. Mediums A (nutrient gelose with bouillon plus 20 parts in 1,000 of PTV peptone), B (same medium with the addition of 10 parts in 100 of defibrinated blood) and C (PTV peptone gelose, 20 parts to 1,000, without bouillon) behaved in an identical manner with a number of colonies of the same order after seeding dilutions limited to 10^{-5} and 10^{-6} with a density analogous to the density of P. pestis and observing the same technique.

2. General characters of the cultures.

Rapidity of cultivation.

It is analogous, according to Delbanco (1896), to the cultivation rapidity of Escherichia coli. Malassez and Vignal's bacillus, on usual culture mediums, rapidly yields a macroscopically visible culture, detectable in liquid medium (in peptone water perhaps better than in plain bouillon), commencing with the sixth hour at 37° and at 28°, whereas the colonies on gelose are visible to the naked eye commencing with the tenth hour of incubation at either one of those two temperatures.

The development is still faster than the growth of Yersin's bacillus (Wilson and Miles, 1955). This rapidity of the cultures, especially on leaving the organism, has a definite orientation value: "every microbe that grows rapidly on gelose on leaving the organism is not a plague bacillus" (G. Girard).

Alkalization of the mediums.

The tendency of Malassez and Vignal's bacillus to alkalize the mediums has been noted for a long time (Klein, 1900; Korobkova, 1929; Dujardin-Beaumetz, 1934; etc.) and some writers proposed using this character for differentiation with Yersin's bacillus (Jurkevitch, 1911). Thus, the medium proposed by Nikanoroff in 1927 shows up the greater speed of alkalization produced by Malassez and Vignal's bacillus. It is composed of Martin's bouillon previously fermented with yeasts in order to eliminate all sugar from it, gelose 2 parts in 100, adjusted at pH 5.7 and with the addition of methyl green 0.07 parts in 100 or phenol red (or cresol) 0.01 parts in 100. When seeded in striae on such a medium, Malassez and Vignal's bacillus culture, becoming more and more abundant, produces a rapid change, whereas the Yersin's bacillus culture is more meager and does not produce a change before 5 to 7 days.

Phenolphthalein bouillon (Zielieczky, 1902) or bouillon without sugar with thymol blue proposed by Nikanoroff (1927) and taken up by Schütze (1929) give similar results.

Korobkova (1929) specified that alkalization is always preceded by acidification, appreciable if the initial pH is 6.4, insignificant or zero if the initial pH is 5.8 to 6.0. Then the alkalization increases constantly, reaching its maximum by the 30th to 35th day.

A daily comparison of the pH in the cultures of Yersin's bacilli and Malassez and Vignal's bacilli shows that, in the latter, alkalization is clearly faster, especially if the initial pH is 5.8 to 6.0 and during the first week of cultivation. After this period of time, the differences in pH between the two cultures decrease progressively and the final pH is identical for both germs toward the 30th to 35th day.

Preston and Maitland (1952) confirmed the initial acidification point out by Korobkova and ascertained that the changes in pH are less pronounced at 22° than at 37°, with a more rapid cultivation at this last temperature.

Pigment.

No strains of Malassez and Vignal's bacilli have ever been pointed out as producing a pigmentation, except for cultures made on potatoes.

Odor.

Although some writers (Gate and Billa, 1928; Winkle, 1955) point out that they did not notice any special odor given off by their cultures, others take it in account. Thus, according to Lucet (1898), Malassez and Vignal's bacillus "emits special volatile products that impart to the cultures, whatever they may be, a very particular nauseous odor of putrefaction". Courmont and Panisset (1914) likewise find a "disagreeable" odor in cultures in bouillon, milk, gelatin and coagulated serum. Ravaglia (1932) mentions an odor "resembling somewhat the odor of colibacillus" in one strain. According to Preisz (1894) "cultures that are a bit old give off a rather strong bad odor that is more characteristic than the morphology of the culture".

Dujardin-Beaumetz (1934) describes this odor as "disagreeably urinous". Totire-Ippoliti (1916), Sanfelice (1926) and Barsini (1935) speak of an "alliaceous" odor close to the one described by Vincenzi (1890-1909) in connection with Bacillus opale agliaceo. Six strains studied by Hendriksen and Jyssum in 1961 emitted "a strong odor, disagreeable at times, quite different from the odor of Pasteurella multocida".

This character is far from being constant, but, as M. Piechaud and we have remarked several times, certain strains give off an odor that is difficult to define but frankly disagreeable.

Specific gravity of the cultures.

Stiegell (1908), who measured the specific gravity of the cultures of various bacterial species, gives the figures 1.177 and 1.215 for cultures of Malassez and Vignal's bacillus on gelose, 80 and 40 days old, respectively.

3. Characters of the cultures on solid mediums.

Gelose.

Phase S is the one under which Malassez and Vignal's bacillus is most usually found. Colonies of this type, developed in 24 hours at 37°, are small in size, not over 0.25 to 1 mm in diameter, round, smooth and slightly convex, transparent and clear at first, sometimes iridescent with a bluish reflection (Preisz, 1896). The edges are sharp and even. They become progressively opaque with a whitish reflection at the same time as the center rises and appears to be more refractive than the periphery. After 48 hours the colonies double in size and are frankly opaque.

Growth is always slower at 22°. Colonies developed at this temperature are more mucous and more moist. The surface is smooth or slightly granular (Knapp, 1959).

At room temperature the growth is appreciably slowed down; nevertheless after 48 hours the cultures are identical to the ones developed at 37° (F. Kauffmann, 1933).

Although this is the aspect of the germ colonies in phase S, the appearance in cultures at 37° of dry, flat colonies, with a slightly granular, dull or shagreen-like surface, while the edges lose their evenness, announces the R-dissociation.

Colonies of Malassez and Vignal's bacillus never present the viscous consistency so frequently found in strains of Pasteurella pestis that have recently left the organism (Eastwood and Griffith, 1914; Devignat, 1954).

Serum gelose and ascitic gelose.

On serum gelose and on ascitic gelose, the colonies are scarcely more volumineus than on plain gelose and attain a diameter of 2 to 3 mm by the second day. Clear and transparent during the first 24 hours, they become whitish or grayish after 48 to 72 hours.

Although Baumann (1927) obtained colonies that were a bit larger by adding serum to the gelose, Wilson and Miles (1955) rightly deny any influence by the serum.

Gelose with blood.

Growth was probably better on gelose with rabbit blood than on serum gelose, according to Seal (1951), while Knapp (1959) and we found no difference between the two mediums.

On gelose with horse or rabbit blood, the colonies are more compact than on plain gelose, according to Topley (1955). That is chiefly true for colonies developed in 24 hours at 18°, and at 28° they are larger than the ones developed at 37°. They reach a diameter of 2 to 3 mm after 48 hours.

According to Devignat (1954), "at 20°C and at 30°C, all the strains of Pasteurella pseudotuberculosis and of Pasteurella pestis develop at different speeds without this diversity being able to be attributed either to the species or to the variety or to the virulence. Nevertheless, if the culture is made on the surface at 37°C, it is observed that the growth speeds and the aspect of the various strains become uniform, at least for 24 hours. After 48 hours at 37°C, four

out of five strains of Pasteurella pseudotuberculosis become grayish and different from Pasteurella pestis".

In the first 24 hours the colonies are clear and transparent; they become opaque, grayish or whitish after 48 to 72 hours. A butyrous aspect of the colonies was observed by Mathey and Siddle (1954). The action on the red corpuscles will be considered farther on.

Coagulated serum.

Growth is always less good on coagulated ox serum than on gelose (Baumann, 1927; Wilson and Miles, 1955). The colonies are not visible to the naked eye before 36 hours and never are greatly developed. Even after one week they do not exceed 0.5 to 1 mm. Very convex, white or opalescent, they have a glossy appearance with a visible reflection when light touches the surface lightly. They do not become yellow slowly as is sometimes the case with colonies grown on gelose.

According to Pfeiffer (1889), Poppe (1928) and Seifried (1937), sheep serum produces better cultures than ox serum or human serum.

Dorset's medium.

Here the aspect is similar to the one obtained on coagulated serum (Moss and Battle, 1941). Both mediums are not liquefied.

Pergola's S.E.T. medium.

This medium, composed of serum, egg yolk and potassium tellurite, has also been used, primarily by Italian writers (Dessy, 1925), with the same results as with the preceding one.

Gelatin.

Colonies appear after 24 to 36 hours on gelatin in a Petri dish incubated at 18° to 20°. They are extremely tenuous and reach the volume of a pin-head only after 48 hours. They are uniformly round, shiny, whitish, and project above the surface of the medium (Megnin and Mosny, 1811). That never is liquefied.

Parietti (1890), Preisz (1894) and Delbanco (1896) observed an opacification of the gelatin in the vicinity of the colonies that are surrounded by a whitish or bluish ring.

Pfeiffer (1889) pointed out the formation of small needle-shaped crystals around colonies on gelatin. According to Parietti (1890) and Delbanco (1896) the same formations may be observed on the butt of gelatin inoculated with a central puncture. Preisz (1894), who pointed out the production of more voluminous crystals deep down in cultures on plain gelose, denies the formation of crystals in gelatin and removes the value that Pfeiffer attached to it.

In the bottom of gelatin seeded by a central inoculation, growth is filiform, confluent at the top, discrete deep down and does not involve liquefaction. According to Knapp (1959), growth is faster in gelose-gelatin than in plain gelose or in glucose gelose.

Potato.

Although a very few writers (Swellengrebel and Hoesen, 1915) did not obtain any development of Malassez and Vignal's bacillus on

a potato, most of them state that this type of culture is possible (Pfeiffer, 1889; Zwick, 1908; Boncinelli, 1932; etc.), but is always very meager and most frequently very slow (Pfieffer, 1889; Nocard and Masselin, 1889; Klein, 1889; Zagari, 1889; Megnin and Mosny, 1891, etc.) in the form of a glossy film that is first colorless or whitish, then yellowish or chamois-colored and, much later, definitely brown (Lucet, 1898; Zlatogoroff, 1904; Schutze, 1928; Barsini, 1935, etc.).

According to Courmont and Panisset (1914), the colonies, pale yellow at first, become brownish-red at the same time as the potato itself is colored a very dark green.

In spite of this opinion and in spite of the opinions of Parietti (1890), of Preisz (1896) and of Dujardin-Baumetz (1934), the pigmentation never extends to the entire medium but remain limited to the culture.

The growth on potato was better at room temperature for Delbanco (1896) and also it was possible only around 20° according to Brigham and Rettger (1935). According to Bergey (1957), it was barely visible before the seventh day. In fact, the culture, more abundant at 37° than at 18°, is visible commencing with the second to third day.

For Dujardin-Beaumetz (1934), the growth on potato depends much on the degree of natural acidity of the tubers. Although, according to Preisz (1894), the bacillus grows as well on a definitely acid potato as on an alkaline potato, most of the writers recommend the latter (Pfeiffer, 1889; Poppe, 1928; Decouze, 1934; Cecarelli, 1950).

Dujardin-Beaumetz compared the limited culture of Malassez and Vignal's bacillus with the complete absence of culture of Yersin's bacillus on potato. There is too much unevenness in the development of Malassez and Vignal's bacillus on this medium to be able to retain this criterium. Besides, the tests that we made yielded, according to the varieties of tubers used and according to the season, results that were too disparate to enable the slightest conclusion to be drawn from them.

Involution forms were frequently observed on this medium (Sacerdotti, 1905; Zwick, 1908).

Leifson's medium (desoxycholate-citrate-agar).

Thal and Chen (1954) and Baltazard and colleagues(1956) proposed this culture medium to differentiate Malassez and Vignal's bacillus from Yersin's bacillus. According to Thal and Chen, after 48 hours at 37° Yersin's bacillus cultures are meager; the colonies, reddish in color, do not exceed the size of a pin-head and the medium is unchanged, whereas the colonies of Malassez and Vignal's bacillus are larger in size, opaque and yellow in color, as the whole medium turns in general.

Knapp (1959), when he was comparing the culture rapidity of Malassez and Vignal's bacillus on plain gelose, gelose with blood, Endo's gelose, and desoxycholate citrate agar at 22° to 37°, found a slight inhibition of the cultures with the last-mentioned medium.

At 22° the colonies are smooth, clear and transparent during the first 24 hours; then they become slightly cloudy and yellowish-brown after 48 to 72 hours. The medium itself is not modified, counter to Thal and Chen's observations. At 37°, the cultures tend toward an R-dissociation that, at this temperature, was more frequent on this medium than on gelose with blood or on Endo's medium (Knapp, 1959-1960).

Although Thal and Chen believe that Leifson's culture medium

facilitates the isolation of Malassez and Vignal's bacillus when using contaminated material, Knapp (1959) believes that it is not suitable for isolation when human stools are used. Nevertheless, Daniels (1961), who made coprocultures systematically on different mediums, considers that the best results are obtained on Leifson's medium, by means of which he was able to isolate the bacillus once in man and nine times in the guinea-pig.

Judging from our experience, Salmonella-Shigella's culture medium gives results comparable to the ones obtained on Leifson's medium, but neither of them, nevertheless, offers the advantages of the medium recommended by E. J. Morris in 1958.

MacConkey's medium.

On this medium, utilized originally by Rosenwald and Dickinson (1944) at the time of an epizootic in turkeys, Malassez and Vignal's bacillus produces, according to these writers, colonies that are strictly analogous to colonies of Salmonelleae. Although for some writers (Topley and Wilson, 1955) colonies grown on MacConeky's medium are meager and confluent and are no longer transplantable after 48 hours, culture on it is, on the contrary, satisfactory and viable, according to Henriksen and Jyssum (1961). The absence of development P. septica on this medium was a good means of differentiation of Malassez and Vignal's bacillus and Yersin's bacillus, for Topley and Wilson (1955)

Wilson Blair's medium.

According to Knapp (1959) and Percebois (1961), M. Piechaud probably did not obtain cultures on this medium with the strain that he isolated in man in 1952. In fact, Piechaud's article does not mention the use of this medium.

At any rate, it is difficult to cultivate Malassez and Vignal's bacillus on it: Knapp (1959-1960) did not obtain visible cultures after 48 hours at 37° and at 22° he only obtained, after massive seeding, a very meager culture with a greenish or chestnut coloration of the culture medium.

Endo's gelose.

Culture is good at 22° on this medium. The colonies that at first are flat, clear, transparent then slightly cloudy, attain in 48 hours the same size as on plain gelose or on gelose with blood and do not produce any modification of the medium. At 37°, on the other hand, the culture is slightly wrinkled and tends toward dissociation.

Lactose gelose with bromothymol blue.

On this medium, colonies are small, smooth, moist, opalescent and blue, convex with smooth edges (Moss and Battle, 1941).

Drigalski's medium.

According to Vourloud (1906), Drigalski-Conradi's lactose culture medium lets Malassez and Vignal's bacillus be differentiated from Yersin's bacillus: "On this medium colonies of plague bacilli

are colored red and the entire medium takes on a general yellowish-red tint similar to the coloring of coli. Colonies of pseudotuberculosis rodentium are not colored but the medium takes on a general bluish-green tint similar to the coloring of the typhic bacillus. That is probably an important distinguishing character for diagnosing the plague bacillus and for differentiating it from the pseudotuberculosis rodentium."

Lerche (1927), Baumann (1927), Truche and Bauche (1929), Pallaske and Meyna (1932) confirmed the aspect of colonies on this medium: "Thin, barely visible, bluish, round, after 24 hours; they are more blue and more round, after 48 hours, and become darker and lose their transparency." (Truche and Bauche).

Orskov and Kappus (1930) recommended the addition of 1 part in 100 of sucrose to this medium on which colonies were then dark blue.

According to our own experience, Malassez and Vignal's bacillus is easily grown on Drigalski's medium at 37°, 22° and 18° and yields moist, glossy, bluish colonies that are more clear and more whitish than the medium itself. The appearance of R-colonies is more frequent at 37° than at 22° and especially than at 18°.

Kristensen's lactose gelose.

Colonies are easy to grow in it at 28° and at 37°, more slowly at 18°. They are white, opaque, convex and glossy. Their confluence produces a rapid alkalization of the medium.

Salmonella-Shigella's medium.

Here the development of colonies is better at 28° than at 37° or at 18°. Colonies in this medium are whitish, glossy, opaque, with a slight rose reflection. Dissociation, constant at 37°, is much more rare at 28° in this medium.

This culture medium gives good results for isolating the bacillus in stools.

Eosin-methylene blue gelose.

Colonies appear a bit faster at 28° and take on a bit larger size in comparison with cultures incubated at 37° and 18°. After 24 hours, they are opaque and whitish, sometimes slightly rose, and on the second day take on a deep violet coloration, without a metallic reflection.

The appearance of R-forms at 37° on this culture medium is more rare than on Leifson's medium.

Meyer and Batchelder's (1926) gentian violet medium.

On this medium, derived from Drennan and Teague's (1917) and containing 0.025 parts in 100 of sodium sulfite and a concentration of gentian violet that varies according to the bacteriostatic effect desired with regard to Proteus, P. septica and other contaminants, colonies of Malassez and Vignal's bacilli display a coloration that is restricted to their periphery, whereas colonies of Yersin's bacilli are only colored in their center. These results were partially confirmed by Bezsonova (1929) and Kuranchi (1951). According to the

latter, if the growth of P. septica is effectively delayed 50 to 60 hours and is even completely inhibited by incubation at 30°, pigmentation of the colonies of Malassez and Vignal's bacilli may be uniform and not exclusively localized at the periphery.

4. Characters of cultures in liquid mediums.

Bouillon.

Pfeiffer (1889) seems to have been the first to point out the clearness of cultures in bouillon, with the production of flakes and deposits.

Lucet (1898) gave the following very complete description of cultures of Malassez and Vignal's bacillus in bouillon: "It grows abundantly when it is seeded in beef bouillon with the addition of peptone and salt. In twelve hours it causes a pronounced turbity that increases subsequently still more and, in 24 hours, forms, on the sides and bottom of the culture flask, a whitish deposit that is distributed uniformly or in the form of large piles. This deposit, that is little adherent to the sides of the container, is easily set in motion by agitating and the lumps are broken up. Then the cultures assume a very special muddy appearance. At rest the deposit forms again and the medium is cleared up somewhat, remaining at the same time, nevertheless, still quite cloudy until the time, quite a while later, when all growth ceases and when the microbes finally fall to the bottom of the container in the form of an extremely abundant pile, still lacking in adherence. At this time the bouillon recovers its clearness.

Moreover, toward the fifth day in the incubator at 37°, or later at a lower temperature, the surface of the nutrient liquid is covered with rather thick, more or less numerous, whitish films. These films, not very resistant, easily dissociated, resembling greasy droplets congealed on a cold liquid, begin to appear either on the edges or in the middle of the free surface. They spread out little by little, touch each other, agglomerate and form a superficial membranous skin entirely covering all the liquid that then loses a bit of its cloudiness. But this membranous covering is not very consistent. The slightest agitation breaks it up and reduces it to fragments that fall to the bottom of the culture flask. Nevertheless, it forms again very rapidly under the same conditions and this continues until the nutrient medium is exhausted, becoming, however, each time less and less thick.

"Sometimes this skin is not continuous and exists only in the form of small islands, varying in size, that float on the surface of the substratum. At other times it is replaced by a simple ring adhering to the sides of the container, at the level of the points of contact of the bouillon. Finally, it usually does not form in cultures made below 20°, or forms very slightly in them."

Delbanco (1896), Saisawa (1913), Messerschmidt and Keller (1914) observed the homogeneous cloudiness, but always slight, of cultures in bouillon.

According to Klein (1906) and according to the Reports of the Plague Commission in India (1908), the turbidity is not homogeneous, while according to Zlatogoroff (1904), Petrie and Macalister (1911), Poppe (1913), the bouillons remain clear. These differences were

found by Kakehi in his comparative study in 1916: "Although the growth of the germ in bouillon is not very energetic, there is no strain that does not produce a certain amount of turbidity that decreases in time and disappears entirely sooner or later. With certain strains the cloudiness disappears in a few days, whereas with others it persists for some time".

Schütze (1928) was the first to have demonstrated that the S-variants produce a diffuse cloudiness with the secondary formation of a ring then of a film, while the R-variants yield a flaky culture with a more pronounced skin, an abundant deposit and, sometimes, the complete absence of turbidity.

Knapp (1959) confirmed that the S-forms produce a uniform cloudiness at 37° and 22° in peptone bouillon with macerated meat. This cloudiness is always more pronounced at 37° than at 22° during the first 24 hours. Clarification with the formation of a deposit and the appearance of skin occurs after two or more days.

The R-forms that grow faster and more abundantly at 37° than at 22° grow without clouding the medium with a rough or flaky deposit.

The intermediate forms first produce a diffuse cloudiness with a deposit and then subsequently a clarification of the culture medium (Boquet, 1937).

Leroche (1926) believed that he was able to differentiate avian strains from rodent strains according to the aptitude of the latter to form a folded skin, but his later observations, confirmed by the observations of Truche and Bauche (1929) on strains coming from turkeys, demonstrated the analogous behavior in plain bouillon of all the strains, regardless of the source.

The formation of stalactites, presented in 1897 by Khabrine as peculiar to Yersin's bacillus, is also found in Malassez and Vignal's bacillus, as was demonstrated by Tartakowski (1901), Zlatogoroff (1904), and the members of the Plague Commission in India (1908). This aspect, frequent in the R-forms of Malassez and Vignal's bacillus, therefore, cannot be used to differentiate the two germs (Dujardin-Beaumetz, 1934; Pollitzer, 1954). Haffkine (1897) demonstrated that these products, starting at the surface, are facilitated by the addition of a film of fatty liquid to the medium. It is possible to obtain the same formations after several days of incubation and without any additive, provided that all vibration is avoided in the cultures (Pollitzer, 1954). Skin and stalactites have, in fact an extreme fragility and the slightest shaking of the test tube is sufficient to precipitate them to the bottom.

The addition of serum or of glucose to the peptone bouillon with macerated meat does not improve the culture, but growth is more abundant and multiplication more rapid in tryptone bouillon (Knapp, 1959).

Peptone water.

Peptone water, in a concentration of 10 to 15 parts in 1,000, is an excellent culture medium for Malassez and Vignal's bacillus, provided that certain peptones (P.T.V., I.B.F., bactopeptone Difco) are used, and for Yersin's bacillus. In comparison with cultures in bouillon, the cloudiness developed in peptone water is always slight, especially at laboratory temperature, during the first 24 hours, but after 48 hours the aspect becomes practically identical in these

mediums, and the cloudiness can even be more pronounced at 28° in peptone water than in bouillon.

Just as they do in bouillon, skin and stalactites assume an identical aspect, but the deposit is generally greater.

Peptone water with the addition of 0.5 parts in 100 of glucose (Otten, 1926) is acidified in 7 days up to a pH at 4.6-4.8, while the addition of only 0.05 parts in 100 of glucose produces a realkalization with a pH at 7 to 7.2, after initial acidification. This secondary alkalization of the peptone water with a weak concentration of glucose was proposed as a diagnostic differential between the plague bacillus and P. septica, with the latter reaching a possible pH at 5.1 to 5.5 or 5.8 to 6.1.

According to Zlatogoroff and colleagues (1928), realkalization occurs more rapidly with the R-forms than with the S-forms of Malassez and Vignal's bacillus.

Yeast water and potato water.

Malassez and Vignal's bacillus, like Yersin's develops rapidly in it, as opposed to Pasteurella septica (G. Girard, 1942).

Bouillon with tetrathionate.

Rosenwald and Dickinson (1944) observed the survival of the bacillus in this medium. Knapp (1959) confirmed that after twelve hours in Difco tetrathionate bouillon there was no decrease in the number of germs, whereas there always is a definite numerical reduction in Leifson's bouillon with selenite.

Barsiekov's medium.

After Otten (1926) and Poppe (1927), Kurauchi (1931) made a comparative study of the action of Malassez and Vignal's bacillus, of Yersin's bacillus and of P. septica in Barsiekov's litmus bouillon containing 1 part in 100 of glucose, 0.5 in 100 of sodium chloride, adjusted to pH 7.6 and incubated at 30°, with the following results: Malassez and Vignal's bacillus produces a strong acidification and coagulation of the medium in 24 hours, while P. septica does not cause any modification, and coagulation with Yersin's bacillus occurs later (three to seven days) and is particularly irregular (18 strains out of 29).

5. Mobility.

The mobility of Malassez and Vignal's bacillus escaped many writers for a long time (Malassez and Vignal, 1884; Pfeiffer, 1889; Zagari, 1890; Delbanco, 1896; Zlatogoroff, 1904; MacConkey, 1908; Petrie and Macalister, 1911; Roemisch, 1921, etc.). Their descriptions, without always specifying, however, the observation conditions and the culture temperatures, verify the immobility of the germ.

The first mentions of its mobility were made in 1889 by Grancher and Ledoux-Lebard who observed the loss of this mobility in old cultures, by J. Courmont and by Nocard and Masselin who describe this bacillus as very mobile. Remy and Sugg (1893) compare

Malassez and Vignal's bacillus with the typhus bacillus "due to its mobility, the number and form of its cilia".

Preisz (1894), in view of the preceding descriptions, affirms the immobility of the microbian chains, but recognizes "really some movement" in the isolated bacilli. Parietti (1890), Sacerdotti (1905), Cagnetto (1905) confirm the mobility. Klein (1899-1900) does not observe any mobility in hanging drops but describes one or two short cilia visible after argentic impregnation. Galli-Valerio (1902), in his discussion of the differentiation elements of Malassez and Vignal's bacillus and Yersin's bacillus, insists on the mobility of the latter, contrary to Gordon's (1897) opinion, without, however, reporting any mobility in the former.

Kossel and Overbeck (1902) deserve the honor of having defined the mobility conditions of Malassez and Vignal's bacillus as a consequence of a study by Moronesco (1899) on a germ isolated from milk and "close to Salmonelleae", immobile at 37° and mobile at room temperature. The same difference in behavior according to temperature was observed by Kossel and Overbeck in three strains of Malassez and Vignal's bacillus.

Byloff (1906) confirms this mobility in one strain and verifies the presence of a polar cilium. The influence of temperature on ciliation was again confirmed by Burckhardt (1914), Henschen (1918) and Klasaj (1921) who, although he was unable to observe any mobility, states that he stained a single extra-polar cilium.

These observations, however, are not admitted by everyone: Gotschlich, in the second edition of Kolle and Wassermann (1912), entrenches himself behind the opinion of Preisz, while Lehmann and Neumann (1907), Torre Ippoliti (1915), Kakehi (1916), Lerche (1927), Beck (1928), Poppe in the third edition of a treatise by Krauss and Uhlenhuth (1928), describe Malassez and Vignal's bacillus as immobile.

Arkwright (1927) confirms its mobility at 18°-26°, that is, according to him, a character adequate for differentiation with Yersin's bacillus, at the same time as he demonstrates the existence of a thermolabile flagellar antigen.

These fundamental facts were taken up in the later studies of Schütze (1929), F. Kauffmann (1933), Weitzenberg (1934), P. Boquet (1936), Levinthal (1937), Himmelfarb (1937), Favorisova (1938), Preston and Maitland (1952), Knapp (1956), Klimova (1956).

The mobility of Malassez and Vignal's bacillus must be considered as a fundamental character: there are no immobile strains (Knapp) just as there are no mobile strains of Yersin's bacillus (G. Girard, Pollitzer, etc.). This character, whose importance was emphasized by Favorisova, Arkwright, F. Kauffmann, constitutes in our opinion the surest cultural criterium for differentiation with Yersin's bacillus [See Note], in spite of the practical difficulties emphasized perhaps excessively by Pollitzer.

([Note:] With one single reservation: by bacteriophage action, Brunet (1952) obtained some definitely immobile mutants of Malassez and Vignal's bacillus.)

The most remarkable peculiarity of the mobility of Malassez and Vignal's bacillus is its rigorous subordination to temperature conditions: zero at 37° [See Note], mobility appears toward 30° (Preston and Maitland; Bergey). It is evident at 16°-20° (Favorisova, Arkwright, Levinthal, etc.) or 20° to 23° (Thal, 1954), in practice it is easily observed at room temperature.

([Note:] Although almost all the strains obey this rule, some exceptions have, however, been pointed out. Although descriptions by Marracini (1935) of strains mobile at 36° do not carry any conviction, Arkwright (1927), as well as Weitzenberg (1935) and Wolff (1956) observed some slightly mobile strains at 37°. Knapp (1956) observed, under the electron microscope, some ciliated elements in two strains cultivated at 37°. G. Girard (1953) likewise reports a human strain that is as mobile at 37° as below 25°. Knapp (1959) gives as a supplementary argument in favor of a possible mobility at 37° the presence of anti-H antibodies in rabbits immunized with cultures made at 37° and killed by heating to 56°-58° (P. Boquet, 1937) or with phenol (Knapp, 1956). This is in contradiction with the observations of Preston and Maitland.)

According to Preston and Maitland, the "critical" temperature for variations in mobility is in the vicinity of 30°. At 27°, 80% of the germs are still actively mobile. At 28°, only 50% are slowly mobile. At 31.5° only one cell, very weakly mobile, was found by these writers. At 34° all mobility disappears.

Mobile germs in a culture at 18°-20° rapidly become immobile again when they are brought up to 37°. Preston and Maitland, nevertheless, observed a temporary persistence of the mobility at 37°. This persistence probably did not exceed one to two hours, according to P. Boquet. Inversely, strains cultivated at 37° and then placed at 22° display already after several hours a certain number of mobile elements.

These variations in mobility, easily observable in vitro, have also been noted in vivo. P. Boquet did not find any mobile elements in the peritoneal exudate of a guinea pig that had been infected, 8 to 24 hours before, with germs cultivated at 20°. The loss of mobility, besides, is very rapid in the peritoneal cavity. One hour after the injection of 4 ml of very mobile germs the exudate no longer contains anything but immobile germs.

Conditions of aerobiosis or anaerobiosis have no effect on mobility (Weitzenberg).

The optimum pH for the appearance of mobility is from 7 to 7.3. In a glucose culture medium mobility disappears progresively together with the acidification of the medium (P. Boquet).

According to P. Boquet (1936), Malassez and Vignal's remains immobile at 18°-20° in Martin's bouillon and displays only a slight mobility in this same medium or in plain bouillon with the addition of 10 parts in 100 of horse serum. On the other hand, mobility is evident in germs cultivated on gelose with an agar concentration of 1.5 to 2 parts in 100 that is neutral or slightly alkaline (pH 7 to 7.3), and freshly prepared or in the condensation water from this medium. It is also evident after 8 to 14 hours in Sauton's synthetic medium, containing 2 parts in 1,000 of glucose in place of glycerin.

The addition of serum or of glycerin to the bouillon does not produce any appreciable modification in mobility, according to Weitzenberg. Only Favorisova considers serum as facilitating its appearance.

The property of producing flagella constitues a stable and transmissible character when the vegetation temperature stays around 20°. When it reaches 37°, this property disappears temporarily, manifesting itself again as soon as conditions become favorable again. Nevertheless, if the inhibitory action is prolonged, the temporary character may become permanent, according to P. Boquet who, after he

had kept two strains of Malassez and Vignal's bacillus for several years, was unable to produce the reappearance of their mobility at 20°.

Age could be a cause of definitive immobility in strains preserved in a collection, according to Favorisova, and P. Boquet observed the same immobility only in the R-variants of two old strains. However, we did not observe this ourselves, since we easily demonstrated the mobility of strains that had been preserved for more than 20 or even more than 30 years.

It seems that the preservation conditions play a part equal at least to the part played by age. Weitzenberg ascertained that four-week old cultures, kept at a low temperature, were more mobile than 24-hour old cultures coming from strains kept at 37°.

Contrary to P. Boquet's opinion, for whom mobility was improved neither by successive transplantings nor by preservation at 20°, repeated transplantings in soft gelose or in serum bouillon and cultivation at 18°-20° allow the most mobile elements to be chosen from each strain (Favorisova, 1938; Thal, 1954).

When Korobkova took up again, with Petrova and Salaeva (1960), the study of strains obtained by her in 1937 from Yersin's bacillus subjected to the action of the bacteriophage and whose characters had been sufficiently modified so as to be no longer differentiable from the characters of Malassez and Vignal's bacillus, she ascertained, nevertheless, that these strains remained strictly immobile.

Every strain does not have the same aptitude for giving birth to elements endowed with mobility (Favorisova; P. Boquet), and some writers thought that they could establish differences according to the animal origin of the strains. Strains of human origin were especially considered by some as always immobile. That is why the strains isolated in the classic examples of Albrecht (1910), Neugebauer (1933), Dujardin-Beaumetz, Ballet and Cebron (1938) were described as immobile (with no indication, however, of the culture temperature) and that is why Moss and Battle (1941), Snyder and Vogel (1943), Topping, Watts and Lillie (1938), describe as a fact the immobility, at 18° and at 37°, of the strains that they isolated. Knapp himself (1954) and Knapp and Masshoff (1954) shared this conviction while studying three strains isolated from mesenteric adenitis in man.

In reality, a subsequent examination of these strains was to reveal their mobility. Thus Plasaj demonstrated a ciliation in Albrecht's strain. Likewise we observed the mobility of strains from Dujardin-Beaumetz and colleagues, Moss and Battle, Snyder and Vogel, Topping and colleagues. Knapp (1956), finally, showed that the immobility of the strains was really more apparent than real, related only to the number of flagellate elements that was too small to show up by staining the cilia or with the hanging-drop technique. On the other hand, the use of Bader's method (initial culture in bouillon at 22° followed by massive seeding in soft gelose, 3 parts in 1,000, in Craigie tubes) shows up the mobility of <u>every</u> strain of Malassez and Vignal's bacillus.

Although no doubt about the mobility of human strains must remain, it is not the less true that, at the time of their isolation, their mobility is at times much slower and more difficult to demonstrate than in strains of animal origin [See Note].

([Note:] Weitzenberg, however, reports a strain isolated in a horse ("Pojeziory" strain) the mobility of which was particularly difficult to verify.)

Although the majority of the strains that we isolated from human mesenteric ganglions showed up as mobile commencing with the first isolation, in them and not in others mobility may be late in appearing (M. Piechaud, 1952) and difficult to demonstrate. Wildfuhr (1959) and Beer (1960) share this opinion.

We made the same finding with strains isolated in the monkey, which can also appear immobile at the time of their isolation (Mollaret, Sizaret and Vallee, 1962).

In practice, it is not always easy to prove the mobility (Weitzenberg; Pollitzer; Knapp), and repeated examinations and transplantings may be necessary.

According to G. Girard (1953), "the homogeneous cloudiness of cultures in a liquid medium at 25° C and below has been given as a sign of mobility, in contrast with the aspect of cultures at 37° C like cultures of Pasteurella pestis at every temperature (skin and snowy flakes in a clear liquid). We have seen enough exceptions concerning the plague bacillus according to the culture medium used (peptone water or bouillon), to the incubation temperature and above all to the age of the strains, so as to assign only a relative value to this character."

A simple examination in a hangin g-drop (Favorisova), or on a slide under a cover-glass, of a drop of culture in bouillon may suffice,but G. Girard also advises against it in view of the risk of doubtful interpretation.

Levinthal (1937) suggested the direct observation of colonies, a technique taken up by F. Kauffmann (1933), Weitzenberg (1934) and modified by Himmelfarb (1937) [See Note] and Wildfuhr (1959).

([Note:] Himmelfarb, moreover, recommends, in order to distinguish true mobility from molecular motion, replacing the physiological serum with a 1 part in 100 sublimate solution that suspends mobility and does not alter the brownian movements.)

Seal (1951) advised a culture in soft gelose incubated at a temperature of 25°. This is the technique that we employed because of its simplicity and effectiveness and that G. Girard defined as follows in his project for the unification of methods for differentiating Pasteurella pestis from Pasteurella pseudotuberculosis (1953): "very soft peptone gelose (4 to 5 parts in 1,000 of gelose), pH adjusted to 7.2, distributed on the butt of the tube to a height of 5 to 6 cm. It is seeded by means of a center injection with a stiff platinum wire carrying a 24-hour old culture on slanting gelose. Two tubes are seeded like this, one of which is incubated at 20°-22° C, the other at 34°-37°C. Pasteurella pseudotuberculosis displays all around the seeding streak a cloudiness that has the appearance of a cylinder, in relation to the diffusion of the microbe in the gelose, indicating its mobility at 20°-22°. Pasteurella pestis yields a culture that is strictly limited to the seeding streak. A reading may be taken already after 24 hours, but there is no disadvantage in waiting three to four days, if the germ is growing slowly."

Devignat (1953) suggested a complex "urea-inulinmobility" medium, including 0.5 parts in 100 of agar and 11 parts in 100 of gelatin.

Preston and Maitland use Craigie tubes that they advise, moreover, sealing in order to avoid desiccation of the gelose.

These cultures in soft gelose are not free from criticism, according to Pollitzer (1954) for whom the mobility of Malassez and

Vignal's bacillus must be distinguished from the tufted appearance that Yersin's bacillus may display in this medium after several transplantings. Therefore, in addition to using the gelose culture medium, it is always necessary to resort also to direct examination in the fresh state and to staining the cilia after cultivation in a liquid medium inoculated at 18°-20° or between 20° and 30° (Knapp, 1960). The choice of the culture medium assumes a particular importance in this research. Knapp (1959) ascertained that, although most of the germs in strains cultivated in plain bouillon have three to six cilia, elements provided with only one to two cilia are found only when the cultures are made on gelose.

Favorinova (1938) advises the use of very soft gelose (0.15 parts in 100) or of serum bouillon, and Wolf (1956) advocates the examination of the condensation water from tubes of slant gelose (1 part in 100) that has been incubated for 18 hours at 22°.

Various techniques for staining the cilia have been recommended: some writers suggest Rhodes' (1958) technique; Weitzenberg (1934-1935) used Zettnow's (1891); Preston and Maitland (1938) advise Conn and Wolfe's (1938); Gunnison and colleagues (1951) and Wolf (1956) suggest Leifson's (1930) modified; and Knapp (1959) advises Sous' (1948) technique.

The number and the arrangement of the flagella have been subject to controversy for a long time. Klein (1899), after agentic impregnation, described one to two polar flagella. Byloff (1906) described one single short cilium that was not as long as the bacillary body. According to Byloff, the shortness and the singleness of this organ accounted for the slight mobility of the germ. According to Burckhardt (1914), most of the elements are aciliated, only a few germs have from one to three short, peritrichous cilia, rarely four. Plasaj (1921) observed only one cilium, growing between the pole and the equator of the germ and several times as long as the microbian body. Levinthal (1930) described an unusual type of ciliation, with a single cilium or a tuft of cilia growing at one, and sometimes at both poles. In many germs the cilium is probably not inserted at the pole itself but alongside or quite some distance away. In amphitrichous strains, the cilia are growing in a diagonally opposite position. Weitzenberg (1933) finds, in general, two cilia, sometimes four and five and even, in one case, six cilia. The extrapolar position is the most frequent, according to him. If the germ is monotrichous, the location of the cilium is lateral. In germs that have two cilia these are located on either side of the bacillary body, opposite each other, or diagonally to each other. The long forms may appear peritrichous. The tufts of cilia described by Levinthal are exceptional for Weitzenberg. Boquet (1937) found, out of twelve strains, only one polar cilium and rarely four to five extra-polar cilia in most of them, a description taken up by J. Dumas (1958).

Since Preston and Maitland's (1952) studies and above all since Knapp's (1956-1959) it appears to be well established that Malassez and Vignal's bacillus has on the average three to six cilia, located peritrichously. Wolf (1956) found an average of two to five and Bergey (1957) counted from one to six.

Although electron microscopy did not enable Parnas (1956) to establish a ciliation, Knapp (1956), by using the same technique as well as Sous' stain, was able to state that most of the strains cultivated in plain bouillon at 22° had three to six peritrichous cilia and

very exceptionally only one to two cilia, located in that case in juxtapolar position. In the same strains cultivated this time on gelose with blood, Knapp no longer found anything but germs provided with only one to two cilia and three very exceptionally.

Although the influence of temperature on the mobility of Malassez and Vignal's bacillus is evident, its mechanism remains unknown. It is not beside the point to recall that this is a question of a phenomenon that does not properly belong to Malassez and Vignal's bacillus. The slowing down (Ch. Nicolle and Trenel, 1902) or the suppression (Braun and Schaffer) of mobility under the influence of physical or chemical factors was observed a long time ago, and, especially, the influence of temperature on mobility was already noted in various species, not only by Mironesco (1899) as we have seen, but also by Ferrier (1895), Migula (1900), Matzuschita (1901), Nicolle and Trenel (1902), Heim (1911), Burckhardt (1914), etc. More recently, Morrison (1961) observed in his strain "8196" of Escherischia coli, aciliated and immobile at 37°, the formation of cilia and the appearance of a mobility after incubation at 20°. Therefore, it is a question of a phenomenon that does not concern exclusively Malassez and Vignal's bacillus and whose mechanism has not yet been elucidated.

Arkwright tried to see in the immobile form of Malassez and Vignal's bacillus an adaptation to parasitism in mammals similar to the adaptation that was observable in Salmonelleae. This finalistic interpretation does not give us any information at all even on the mechanism of this adaptation.

Preston and Maitland (1938) advanced several hypotheses. According to them, the appearance of flagella at 20° and their disappearance at 37° could be due either to the establishment of different physicochemical conditions (especially with regard to the medium's pH at various temperatures), or to the production of an enzyme capable of lysing, only at 37°, the flagella themselves or some substance indispensable for their synthesis, or to a mutation followed by selection, under the influence of temperature, of the only mobile elements, or, finally, to the existence of different metabolic processes at 37° and at 22°, a phenomenon that is close to the findings made by Hills and Spurr in 1952 on the acid deaminases of Yersin's bacillus. Preston and Maitland's experiments threw out the first three hypotheses and it seems that research must be directed toward establishing the difference in the metabolic processes at 22° and at 37°. In fact, the disappearance at 37° of the mobility of strains of Malassez and Vignal's bacillus cultivated at 22° does not seem to be linked to the loss of flagella but to the establishment of conditions unfavorable to a group of metabolic phenomena accounted for, in Preston and Maitland's experiments, by the heavy mortality observed at the time the cultures were transplanted from 22° to 37°.

These findings are added to Morrison's on the paralyzing action of incubation at 37°, in the presence of chloramphenicol, on the cilia of Escherichia coli.

The hypothesis of differences at 22° and at 340 in the metabolic processes concerning the mobility of Malassez and Vignal's bacillus is, moreover, in perfect agreement with the fact that, not only mobility, but also a number of other characters are likewise influenced by the culture temperature. This had been known for a long time, in so far as the virulence of the germ is concerned. Appreciable differences were also noted in its behavior at different temperatures in the presence of sodium citrate, urea and sodium malonate (Mollaret, 1961).

6. Dissociation.

Saisawa (1913) and Poppe in the same year were the first to report dissociation phenomena in Malassez and Vignal's bacillus. Three years later, Kakehi described three forms of colonies: transparent, shiny and slightly bluish "A" colonies that he considered as the normal form, clouding the bouillon with slight involutions; opaque, grayish-white "B" colonies that do not cloud the bouillon but form flakes, a deposit and a skin; and mixed "C" colonies closely resembling the preceding ones but with a bluish peripheric ring, clouding the bouillon uniformly and producing flakes and skin.

Then various types of colonies are described by Arkwright (1919), Roemisch (1921), Miller and Gladky (1927) and especially Zlatogoroff and Mogilewskaia (1927-1928) who distinctly individualized the characters of the S- and R-variants, their biochemical and serologic identity and their uneven virulence. These facts were taken up by Haupt (1928) who described three aspects of the bacillus in bouillon: homogeneous turbidity, homogeneous turbidity with flakes and stalactites, and isolated flakes and stalactites; then by Pokrovskaia (1930) who, in addition to the R- and S-forms, described an intermediate "O" type; finally by Vedder (1933), P. Boquet (1936-1937), Lätje (1941), Knapp (1959), etc.

The smooth and rough variants constitute the two usually observed aspects, with the smooth form being by far the one most frequently found.

S-colonies on gelose are smooth, round, convex, glossy with even edges, uniformly translucent or whitish, whereas the rough colonies are uneven, notched, larger, flat, with a dull, dry appearance, opaque and capable of being picked up with an ease all in one piece.

Colonies that do not answer the description of either one of these two types were described: "mixed" or "C" colonies of Kakehi, "O" colonies of Pokrovskaia, "transitory" colonies of Zlatogoroff and Moghilewskaia, "B" colonies of P. Boquet with a raised central area, surrounded by a flat area with irregular edges, "intermediary" colonies of Knapp, etc.

The S-colonies are easily emulsified in physiological water and the suspensions obtained remain homogeneous after four hours in the incubator (Kakehi, 1916; Pokrovskaia, 1930; P. Boquet, 1937), whereas the R-colonies are difficult to emulsify, have a tendency toward auto-agglutination (Lätje, 1941) and producing suspensions with large flakes. The consistency of the character of the emulsions in physiological water at 37° led P. Boquet to suggest replacing the terms "smooth" and "rough" with "homogeneous" and "agglutinable".

After 24 hours in bouillon at 37° the S-variants, stable, cloud the culture medium uniformly, whereas the R-variants develop into small flakes or stalactites (Pokrovskaia, 1930).

At 20°, in soft gelose, the colonies are fatty and the differential characters of both types are less clear; the R-variant, however, preserves its agglutinability in physiological water at 37°. When they are cultivated at 20°, both variants contain mobile germs (P. Boquet, 1937).

Morphology of the variants.

Morphological differences in the germs in both phases were noted by numerous writers (Kakehi; Zlatogoroff and Mogilewskaia; Pokrovskaia;

Lätje, etc.).

The germs in phase S are thinner and longer (1.5 to 3μ), evenly colored, whereas the germs in phase R are larger, thicker, more intensely and at times more unevenly colored. Only Zlatogoroff and Mogilewskaia describe the germs in phase S as shorter and the germs in phase R as longer and thicker.

According to Kakehi, the formation of chains of ten to 15 elements was characteristic of the rough forms.

Biochemical behavior of the S-and R-variants.

Although for most of the writers (Pokrovskaia, 1930; Thal, 1954, etc.) there is no difference in the biochemical behavior of the S- and R-variants, others report differences that at times are qualitative but most frequently quantitative.

Thus, according to Kakehi, the B-variants (phase R) attack adonitol more slowly than the A-variants (phase S).

Miller and Gladki (1927) described a strain whose S-variant no longer attacked glucose.

Zlatogoroff and Mogilewskaia (1927-1928), while examining the behavior of the two variants, R and S, in the presence of glucose, levulose, galactose, mannitol and ulcitol noted that the attack by smooth strains was always slower. The difference, still appreciable at the 36th hour, disappeared after three days. Zlatogoroff reports a strain whose R-variant alone attacked dulcitol.

In the glucose culture medium, recommended by Otten for differentiating Yersin's bacilli and Malassez and Vignal' bacilli, the smooth variants of the latter produce less acidity than the R-variants. The same is true in Himmelfarb's maltose culture medium.

According to the comparative study of the fermentation speed of glucose, levulose, galactose and maltose made by P. Boquet (1937), the smooth strains are, in the majority of the cases, more active in the presence of these substances than the rough strains and acidification is already clearly marked even in the first hours in glucose and levulose mediums. On the other hand, peptone water with the addition of glycerin is acidified more rapidly by the rough variant than by the S-variant.

Barsini (1935), when he was filtering the two A and B variants of a strain of Malassez and Vignal's bacillus on a bougie, noted that the A-variant (in all likelihood smooth) was easier to filter than the B-variant.

H.O.S. Morin (1943), who applied Robin's reaction (an emulsion of young cultures of gram-negative bacilli in a concentrated solution of sodium or magnesium sulfate produces a definite agglutination only in the case of germs in phase S) to different Pasteurelleae, noted that only the smooth colonies of Malassez and Vignal's bacillus showed this agglutinability. The reaction remained negative with Yersin's bacillus and P. multocida.

The smooth colonies are agglutinated better by serums prepared with homologous or heterologous smooth strains than they are by serum prepared with their own strain of a non-dissociated origin or with the R-variant. Non-dissociated cultures are agglutinated in the same way by a homologous serum or by a serum prepared with one of their S-variants. (Zlatogoroff and Mogilewskaia, 1928). The R-colonies are characterized by the absence or little development of the O antigen

(Thal, 1954).

When it exists, the hemolytic power of the R- and S-variants is identical (Devignat, 1954).

Pathogenic power of the variants.

As early as 1930 Pokrovskaia noted the extreme virulence of germs in phase S for white mice and guinea-pigs, whereas germs in phase R, in the same dosages, are not always mortal or are so only always after a longer period of time. The experimental disease assumes an acute form after inoculation of the S-form and a chronic one with the R-form. Autopsy reveals an efflorescence of necrotic foci in the first case, while the nodules are rare or absent in the second. Only Fertig (1962) obtained an inverse result: the R-variant killed the guinea-pig more rapidly than the S-variant.

Pokrovskaia's results were confirmed by Zlatogoroff and Mogilewskaia and then by Lütje. According to the latter, in the natural disease of the animals the S-form is the one that is isolated in acute cases and the R-form in the course of chronic infections.

Dissociation factors.

Simple aging of the cultures constitutes, as P. Boquet (1937) noted, an important dissociation factor of the germ. According to Thal (1954), some old strains no longer develop except under the R-form.

The composition of the culture medium may also favor dissociation. Thus, according to Zlatogoroff and Mogilewskaia, the quality of the peptone probably determines one or the other type. Witte's peptone favors the appearance of type R, whereas Merck's causes the appearance of smooth colonies.

Kakehi, when comparing the rapidity of appearance and the proportion of variants, appearing in various culture mediums, bouillon, litmus milk and sugar mediums, concluded that glucose, and then mannitol, are the best nutrient factors for dissociation.

According to P. Boquet, the addition of 0.5 to 2 parts in 1,000 of glucose in non-glycerin Sauton's liquid, in which cultures of Malassez and Vignal's bacillus are ordinarily poor, favors the development and the differentiation of the R- and S-types.

According to Knapp (1959), the appearance of the R-variant is especially rapid at 37° on Leifson's medium (desoxycholate citrate agar). It is also favored by using Endo's sulfite gelose.

According to Dujardin-Beaumetz (1934), dissociation is probably not present in strains isolated in hares, guinea-pigs and canaries. Personally, we have never differentiated between the animal origin of the strains.

The tendency toward dissociation varies appreciably from one strain to another. Some of them dissociate easily and produce stable variants, whereas others, more recently isolated, yield unstable variants, according to P. Boquet.

The proportion of colonies of either type varies according to the strains (Zlatogoroff and Mogilewskaia) and according to the age. The number of R-colonies obtained by P. Boquet was larger when it was a question of strains that had been isolated for a long time.

The delay in the appearance of dissociation in bouillon varies from seven to fifty days, according to the strains. It is probably

more rapid in vivo. The S → R transition may be obtained at the eighth generation. The R → S transition, difficult to obtain on gelose, is more easily produced in bouillon and especially in vivo (Pokrovskaia, 1930).

Stability of the variants.

Although the R-variant is observed to be stable in vitro, the stability of the S-variants obtained is a function of the strains, of the culture medium and of age (P. Boquet). Thus this writer demonstrated that, although the variants obtained with some strains are unstable, others, that appeared to be stable on gelose, showed fluctuations according to the culture medium utilized: "In Martin's glucose bouillon, 2 parts to 1,000, or with the addition of 1/10 of normal horse serum, the S-variant produces small flakes, while the R-variant causes a uniform cloudiness in Martin's bouillon with the addition of 1/5 of its volume of Legroux's artificial ascites. On glucose gelose, 20 parts in 1,000, the S-variant provides uneven, flat, opaque colonies, very closely resembling the rough type colonies. All the particular aspects observed on special culture mediums are transitory and disappear to make way for the rough and smooth characters in all their distinctness when a culture is transplanted on the usual mediums (bouillon and gelose). By straining Martin's bouillon every twenty-four hours, the S-variants of some strains are kept in a pure state. But after they are several days old, a dissociation of R-elements is produced. These elements may be isolated easily by means of a sub-culture on gelose. Under the same conditions, the R-variant of the same strains remains stable."

In vivo, the strains inoculated in the animal do not undergo any modifications and are found again in the phase in which they were inoculated, according to P. Boquet, who was able to re-isolate some S-colonies by using organs from a guinea-pig put to death on the third day after intra-peritoneal inoculation of 2 ml of a 24-hour old culture in phase S and of colonies in phase R in another animal inoculated under the same conditions with the R-variant of the same strain.

These results contradict Miller and Gladky's (1927), according to whom, in the course of its passage through the animal, a return to the initial phase is observed in every organ, except in the spleen, and Zlatogoroff and Mogilewskaia's who, after the death of the animals, re-isolated either the homologous phase of the one inoculated, or the other one, or the phase of intermediate aspects. We also made the same observations.

These divergent results may be due to the fact that the re-isolation of the strains was made by P. Boquet in animals put to death three days after inoculation and by Zlatogoroff and Mogilewskaia after complete evolution of the experimental disease and spontaneous death.

Pokrovskaia's (1930) experiments justify this point of view. The inoculation of heavy doses of strains in phase S in mice causes death without the appearance of R-forms, whereas the injection of weak doses produces a slow death and permits the isolation of S-forms as well as of R-forms and of intermediate type colonies. Inversely, the injection of heavy doses in phase R may cause death without the appearance of S-forms and, if weak doses of phase R are inoculated and if death occurs, the transition of the R-form to the S-form is observed.

III — METABOLIC CHARACTERS

1. General metabolic properties.

a. Study of the oxidating enzymatic systems.

Catalase.

Every writer conducted research on this subject obtained a positive reaction with Malassez and Vignal's bacillus as well as with Yersin's bacillus (Kurauchi, 1931; Brigham and Rettger, 1935; Savino and Anchezar, 1939; Macchiavello, 1941; Cecarelli, 1950; Mathey and Siddle, 1954; Devignat, 1954; Topley and Wilson, 1955; Ochi and colleagues, 1956; Bergey, 1957, etc.).

We made the same observation on all our strains of Malassez and Vignal's bacillus and of Yersin's bacillus.

The reaction was also positive with every strain of *P. septica* that we examined, in conformity with the conclusions of most writers (Topley and Wilson, etc.) and contrary to the opinion of some (Kurauchi, 1931).

Oxidase.

Brigham and Rettger (1935) were the first to verify the absence of peroxidase in Malassez and Vignal's bacillus, in Yersin's bacillus [See Note] and in *P. septica*, results confirmed by Steel (1961) with regard to the first two germs. The results were variable with *P. septica*.

([Note:] Only Gordon and McLeod (1928) found a slightly positive reaction with a single plague strain.)

Henriksen and Jyssum (1961) inferred the absence of oxidase in Malassez and Vignal's bacillus, as contrasted with *P. septica*, *P. hemolytica*, *P. pneumotropica*, and *P. tularensis*.

By using Kovacs' technique, by spreading streaks of the culture on filter paper and adding a drop of aqueous solution of dimethyl-paraphenylenediamine hydrochloride, 1 part in 100, we very consistently verified the absence of oxidase in Malassez and Vignal's bacillus and Yersin's bacillus and its presence in nineteen strains of *P. septica*.

Cytochrome oxidase.

Examination for a cytochrome oxidase (Nadi reaction) was negative with all our strains of Malassez and Vignal's bacillus and positive with our strains of *P. septica*.

b. Action of enzymatic inhibitors.

Herbert (1949) seems to have been the first to study the action of potassium cyanide on the growth of Yersin's bacillus. Braun's test was first used on Malassez and Vignal's bacillus by Knapp (1959) who found it positive (absence of culture). Lindermann, Wintsch and Hedinger (1960) made the same observation on two human strains.

We confirmed these results (1961), with 327 strains, none of which, and none of Yersin's bacillus, grew in a culture medium containing 15 ml per 1,000 of a 0.5 parts in 100 solution of potassium cyanide. On the other hand, out of 15 strains of *P. septica*, 8 pro-

duced a culture between the 36th hour and the 48th hour. Steel and Midgley (1962) confirmed the variability in the behavior of this last-mentioned species in a cyanide medium.

c. Dehydrogenase.

Brigham and Rettger (1935) observed that in the presence of sodium succinate methylene blue was decolored by Malassez and Vignal's bacillus, Yersin's bacillus and P. septica; the time for the reaction varied with the species. Thus Malassez and Vignal's bacillus produced a complete decoloration in ten minutes, whereas Yersin's bacillus and P. septica required incubation at 37° to start the reaction and a period of thirty minutes to complete it.

d. Modifications of the oxidation-reduction potential during the course of the culture.

Brigham and Rettger (1935) demonstrated the presence of a reductase in Malassez and Vignal's bacillus and numerous writers studied the reduction power of this germ in the presence of various dyes in the hope of demonstrating a difference in behavior that could be used to distinguish it from Yersin's bacillus.

Megnin and Mosny (1891) were the first to point out the decoloration of gelose with red fuchsin.

The reduction of malachite green, demonstrated by Zlatogoroff (1904) but not observed by Zwick (1908), was used by Petrie and Macalister (1910-1911) who suggested bouillon with malachite green [See Note] as a differential culture medium, a medium used by Kakehi (1916), Schütze (1928), Korobkova (1929), Cecarelli (1950).

([Note:] A confusion occurs frequently with regard to bouillon with malachite green, improperly called by some Loeffler's medium. The real Loeffler's medium with malachite green includes, in addition, lactose and glucose (Trawinski, Zbl. f. Bakt., 1922, 88, 25) and has nothing to do with bouillon to only malachite green has been added that is used in examining for the reduction of this last body.)

In addition to malachite green, Cecarelli verified the reduction power of Malassez and Vignal's bacillus in the presence of Janus green, thionine, methylene blue and neutral red.

Neutral red is reduced, according to Korobkova (1929) and Cecarelli (1950). It is not reduced, according to Zwick (1908), Baumann (1927), Beck (1928), Truche and Bauche (1929), F. Kaufmann (1933), Dujardin-Beaumetz (1934), Barsini (1935), Hauduroy (1953), Devignat (1954), Percebois (1951). According to our experience, Malassez and Vignal's bacillus does not reduce neutral red. We observed this with 172 strains seeded both in a gelose culture medium and in glucose V. F. bouillon, 2 parts in 1,000, in a Hall tube.

Korobkova (1929), who made a systematic study of the reduction power of Malassez and Vignal's bacillus and of Yersin's bacillus in the presence of various dyes in Martin's bouillon with pH 6.8 - 7, with a laboratory temperature of about 28°-30°, noted that both germs reduced, but the first one much more rapidly or more intensely, the following dyes in the order of increasing difficulties: thionine, methylene blue, Janus green, indigo disulfonate, neutral red, malachite green and litmus. Neither one of them reduces Congo red and safranine.

Ivanovski and Sassykina (1930) suggested as a differentiation criterium of the two germs Schardinger's reaction: 0.5 ml of Schardinger's reagent (5 ml of a saturated alcoholoc solution of methylene blue and 5 ml of formaldehyde to 22 ml of water) are added to 3 ml of a two-day old culture suspension in physiologic water (3.5 X 10^9 germs per ml). The whole is put under liquid petrolatum in an incubator at 30°. Malassez and Vignal's bacillus produces a total decoloration in less than one hour, whereas Yersin's bacillus only gives a slow, partial decoloration. This reaction that we used is, in effect, very reliable.

We prefer, nevertheless, to investigate the reduction of methylene blue on cultures in a liquid culture medium, and, rather than in plain bouillon, in milk with the addition of a drop of an aqueous solution with 1/100 of methylene blue per 10 ml tube. Under these conditions, Malassez and Vignal's bacillus produces, already in the first 24 hours, a definite reduction whose translation is different from the one produced by Yersin's bacillus. With the latter reduction always begins in the lower part of the tubes and moves up more or less higher, according to the strains, whereas Malassez and Vignal's bacillus produces a reduction that always appears half-way up the tubes under the form of a white ring (sometimes two) suspended between the surface and the depth of the medium whose coloration remains unchanged.

According to Savino and Anchezar (1939), Costa (1947), Topley and Wilson (1955), Knapp (1959), Malassez and Vignal's bacillus and P. septica reduce methylene blue contrary to Yersin's bacillus. Also according to G. Girard (1953), methylene blue is reduced by Malassez and Vignal's bacillus and not by Yersin's bacillus. In fact, when it was investigated as we have just described, reduction was constant with every strain of Malassez and Vignal's bacillus and of Yersin's bacillus that we examined.

Litmus is not reduced either by Malassez and Vignal's bacillus or by Yersin's bacillus (Devignat, 1954).

e. Reduction of nitrates.

The aptitude of Malassez and Vignal's bacillus for reducing nitrates into nitrites is variously evaluated according to the writers.

For Schütze (1928), Ravaglia (1932), Kelser (1938), Hauduroy (1953), Marracini (1953) (but the authenticity of the strains described by the latter is more than doubtful) it is probably incapable of this reduction, whereas its ability to reduce, variable for Dungall (1931), is affirmed by Knoovalova (1930), Brigham and Rettger (1935), U''janova (1961), Devignat (1953), Knapp (1959-1960).

Out of 533 strains of Malassez and Vignal's bacilli seeded in macerated meat and peptic hydrolysate of pork belly bouillon with the addition of 1 part in 100 of nitrate, we observed reduction in three days at 37° in 530 of them. One strain (468-1) that did not reduce at 37°, reduced, however, at 28° and at 18°. Only two strains (157-1 and 266-1) did not produce any reduction, since the reaction was controlled by the addition of zinc powder.

The reduction of nitrates does not make possible a differentiation from Yersin's bacillus, according to Brigham and Rettger (1935) and especially according to Devignat (1953) who specified that both the ancient and the oriental varieties of this germ were equally

capable of producing the same reduction.

This reduction of nitrates into nitrites by Malassez and Vignal's bacillus, and by some strains of Yersin's bacillus, must not be confused with the production of nitrous acid in culture mediums not provided with nitrate (see below).

f. Tetrathionate-reductase.

Discovered in various bacterial species by Pollock and Knox (1943), this enzyme that catalyzes the reduction of tetrathionate into thiosulfate was investigated systematically by Le Minor, Pichinoty and Coynault (1962) in various gram-negative bacteria. We refer to this study and to one by Pichinoty and Bigliardi-Rouvier (1962) for a description of the techniques utilized.

According to Le Minor and colleagues, who examined 69 of our strains of Malassez and Vignal's bacillus, 23 of Yersin's bacillus and 16 of P. septica, tetrathionate-reductase could not be established except in this last-mentioned species and it is lacking in the first two.

g. Coagulase and fibrinolysis.

Jawetz and Meyer (1944) observed, in virulent and avirulent plague strains, a coagulating activity in the presence of rabbit and guinea-pig plasmas. We did not make the same observation with Malassez and Vignal's bacillus, any more than we were able to detect in it any fibrinolytic activity in the presence of guinea-pig fibrin, whereas Madison (1936) confirmed a pronounced fibrinolytic power in the presence of rat and guinea-pig fibrin with Yersin's bacillus.

2. Action on carbohydrate substances.

a. Action on pentoses, hexoses, disaccharides, trisaccharides, polymerized glucides, polyalcohols and glucosides.

The action of Malassez and Vignal's bacillus on the various carbohydrate substances has been the subject of a large number of studies, some of them devoted exclusively to the study of this germ (Pfeiffer, 1889; De Blasi, 1908; Lerche, 1927; Christensen, 1927; Poppe, 1928; Haupt, 1928; Schütze, 1928-1929; Mørch and Krogh-Lund, 1931; F. Kauffmann, 1933; Tumansky, 1948; Van Dorssen, 1951; Thal, 1954; Marthedal and Velling, 1954; Wilsen and Miles, 1955; Goyon, 1956; Knapp, 1959, etc.), others searching, in a study of so-called fermentation reactions, for differentiation criteria from Yersin's bacillus (Galli-Valerio, 1902-1903; Zlatogoroff, 1904; The Plague Commission in India, 1908; Vourloud, 1908; MacConkey, 1908; Swellengrebel and Hoesen, 1915; Otten 1926; Bishop, 1932; Boncinelli and Aradas, 1933; Uriarte and Villazon, 1935; Wu, Chung and Pollitzer, 1936; Russon, 1939; Savino and Anchezar, 1939; Prado, 1940; Kurauchi and Nagata, 1950; Seal, 1951; G. Girard, 1953; Devignat, 1954; Pollitzer, 1954; K. F. Meyer, 1958, etc.).

From a survey of bibliography on the subject, and from our personal experience extending to 617 strains, it is obvious that Malassez and Vignal's bacillus is capable of producing acidity in a constant manner by using the following bodies: rhamnose, arabinose, xylose, glucose, galactose, levulose, mannose, dextrose, maltose, trehalose, melibiose, amidon, mannitol, glycerin, esculin, argutin, salicin and in an almost constant manner with dextrin and inulin. This acidification is never accompanied by the production of gas (Delbanco, 1896, etc.), although Hauduroy (1953) mentions its possibility [See Note].

([Note:] Gracia Mira's (1945) study, to which Hauduroy refers, reports some very questionable strains, at the very least, that decompose glycerol, maltose, salicin and lactose with gas. The various gasogenic strains that have been described, such as the ones that Nicolle and Sparrow (1928) isolated at the time of the second epizootic observed by them in guinea-pigs in the Pasteur Institute in Tunis and that acidified dextrose, mannitol and maltose with gas, cannot be considered as authentic Malassez and Vignal's bacilli either.)

Lactose, sucrose, melezitose, erythrol, inositol, dulcitol are never acidified by Malassez and Vignal's bacillus, and melitose, arabitol and amygdalin are acidified only very exceptionally.

With sorbitol and adonitol the results are variable.

On the whole, these characters are remarkably stable, and authentic exceptions are relatively rare. Although a number of strains have been described by various authors as having atypical fermental characters, a study of most of them did not enable us to find the indicated discrepancies. Knapp (1959) attributes real exceptions either to temporary variations, or to differences in the culture mediums utilized or the incubation temperatures. We lay the blame, primarily, on the culture mediums (peptone in particular) or on the indicator used, and we consider real exceptions to be very rare.

Acidification of carbohydrate substances by Malassez and Vig-

nal'z bacillus is usually rapid and may be observed commencing with the first 24 to 48 hours of incubation.

With certain strains, and above all with certain sugars in a peptone culture medium, it is possible to observe a color change phenomenon due to secondary alkalization by production of ammonia. Thus Devignat (1961) demonstrated that in peptone water with phenol red containing 2 parts in 100 of inulin the medium turned definitely yellow in 48 hours, then bright red toward the third or fourth day. Snyder and Vogel (1943) observed the same phenomenon when salicin was acidified by the "Spokane" strain.

With the exception of glycerin, whose acidification must be sought in a solid medium, we studied the behavior of Malassez and Vignal's bacillus with the same results in Hiss's medium and in peptone water, 2 parts in 100, using phenol red as an indicator and achieving a final glucide concentration of 2 parts in 100. The choice of the peptone assumes a particular importance in studying the acidification of certain carbohydrate substances, like, for example, salicin and dextrin. The choice of the indicator also is not immaterial, as Devignat (1961) ascertained in connection with the acidification of inulin.

The various sugars are acidified in an identical manner at 18°, 20° and 37°. Nevertheless, some exceptions have been pointed out: the acidification of glycerol is probably weaker and slower at 18°-20° than at 37°, according to P. Boquet (1936). Beaudette (1940) observed, in a strain isolated in a blackbird, that maltose was acidified more slowly at 18° than at 37°. The acidification of esculin, while it is slow, is more evident at 28° than at 37° (Mollaret, 1961).

The S- and R-variants usually show no differences in behavior in the presence of carbohydrate substances. Nevertheless, according to Zlatogoroff and Mogilewskaia (1928), the R-variant probably ferments glucose, levulose, galactose, mannitol and dulcitol more rapidly than the S-variant. The opposite was observed by Kakehi (1916) with respect to adonitol and especially by P. Boquet (1937) with respect to glucose, levulose, galactose, maltose. Only glycerin was acidified more rapidly by the R-variants.

The individual reactions of strains of Malassez and Vignal's bacillus are, on the whole, stable and characteristic (Knapp, 1959), just like strains of Yersin's bacillus (Pollitzer, 1954). All the strains preserved in a collection in the Pasteur Institute by Truche, Dujardin-Beaumetz, G. Girard, and then by us, some of which for more than thirty years now, have not shown, in subsequent examinations, any difference in their behavior in carbohydrate culture mediums in comparison with the original studies.

Differences between the fermental characters of a strain on isolation and after subsequent transplantings have been observed only very exceptionally. Gate and Billa (1928) report a strain that fermented only levulose at the time of its isolation but whose subsequent transplantings fermented maltose, mannitol, glucose, xylose and glactose. On the other hand, Mathey and Siddle (1954) isolated a strain in a turkey that, commencing with the isolation, acidified not only arabinose, glucose, maltose, mannitol, levulose, rhamnose, trehalose and xylose, but also sucrose; this last-mentioned character disappeared after several transplantings. In some cases, transplanting in the animal seemed to strengthen the glucosidolytic power to

some strains, like Beaudette's (1940) or the one that we isolated in a hare (1961) and that, although it did not acidify esculin initially, gave us, after being transplanted in the rabbit and after action of the bacteriophage, cultures that acidified this glucoside.

Action on pentoses.

Rhamnose: It would not be possible to consider the action of Malassez and Vignal's bacillus on rhamnose without comparing it with the action of Yersin's bacillus in view of both the dogmatic and the practical importance of the behavior of both germs with respect to this body.

Rapid acidification of rhamnose culture mediums by Malassez and Vignal's bacillus is a rule that has no exception (G. Girard). Although, according to Stephan (1941) and Brunet (1952), Bitter and Dolomonova mentioned strains that do not ferment rhamnose, all the authors are unanimous in confirming the rapid acidification of rhamnose culture mediums. The strains described by Hässig, Karrer and Pusterla (1949), Terni and Kircheis (1950), Marracini (1953), as not acidifying rhamnose show, moreover, too many abnormal characters to enable us to retain them as authentic Malassez and Vignal's bacilli. Ippen and Stoll (1959) described a strain isolated by them in a deer as acidifying rhamnose weakly, but the sub-culture of this strain, transplanted in a rabbit that those authors sent us, acidified rhamnose, without any possible doubt, in 24 hours.

Some strains might possibly produce a secondary alkalization after an initial acidification, like the "Spokane" strain of Snyder and Vogel (1943). The sample of that strain that we examined did not produce this secondary alkalization for us, a fact that probably does not take away anything from the value of the first acidification.

Therefore, the fermentation of rhamnose must be considered as an essential character of Malassez and Vignal's bacillus, and the rapidity [See Note] of this fermentation is one of the best criteria for differentiating it from Yersin's bacillus.

([Note:] The possibility of a late change has been pointed out: 5 days (Thal, 1954); 8 days (Geurden and Willems, 1940).

The differences in behavior of the two germs with regard to rhamnose were originally pointed out by Vourloud (1908), Swellengrebel and Hoesen (1915), and then were confirmed by D'Aunoy (1923) and Schütze (1928). Bezsonova (1929) and Bezsonova and Lochow (1930), who compared the behavior of ten strains of Malassez and Vignal's bacillus and 160 strains of Yersin's bacillus in a rhamnose liquid culture medium, confirmed the production of acidity in 24 hours by the first-mentioned, whereas not one strain of the second-mentioned bacillus caused modifications after three weeks of observation. The culture medium recommended by Bezsonova (peptone litmus water containing 0.5 parts in 100 of peptone, 0.5 parts in 100 of sodium chloride and 1 part in 100 of rhamnose) met with great favor from then on and a test for fermentation of rhamnose was considered as the only sure biochemical criterium for distinguishing between the two germs (Boncinelli and Aradas, 1933; Uriarte and Villazon, 1935, etc.). Nevertheless, Korobkova (1936), when studying 32 strains of Yersin's bacillus that had been preserved for more than twelve years, observed the acidification of rhamnose by four of them and concluded that Bezsonova's culture medium could no longer be considered as a

sure differential medium. Then Berlin and Borsenkov (1938) showed that mutants capable of acidifying rhamnose could appear in cultures of Yersin's bacillus after more than six days in a rhamnose medium and they limited the period of observation of Bezsonova's medium to a maximum of two to three days, in order for it to retain its full value. Tumansky (1939) confirmed this late acidification (five to fourteen days) of the rhamnose with 84 out of 260 strains of Yersin's bacilli and associated this positive rhamnose character of certain plague strains with their age. Krainova (1939) specified that only plague strains from southeastern Russia, China and Mongolia, that did not ferment rhamnose initially, became capable of doing so after successive transplantings in a rhamnose medium, and acidification could even be evident in the first 24 hours, as in the cas of Malassez and Vignal's bacillus. There was, therefore, a relationship between the geographic distribution of the plague strains and the fermentation reactions of rhamnose analogous to those that Kurauchi had observed in connection with the fermentation of glycerol. Krainova was able to establish a relationship between the aptitude for acidifying glycerol, making the acidification of rhamnose a special property of the strains that ferment glycerol, and, for this reason, connected plague strains from southeastern Russia, Mongolia and China, that fermented glycerol and were capable of acidifying rhamnose after succesive transplantings in a rhamnose medium, with Malassez and Vignal's bacillus, in contrast with strains coming from India or from Madagascar that did not have, right at their origin, the property of fermenting glycerin.

More evidence was still to appear that was of a nature to disturb the value of Bezsonova's medium. Thus Bezsonova herself described five "wild" strains of Yersin's bacillus that fermented rhamnose not after transplanting but as soon as they had been isolated. Devignat (1954) likewise observed a delayed acidification and Brygoo and Courdurier (1955), while studying the behavior of 105 strains from Madagascar with respect to rhamnose, found two of them that were capable of using this body at the very beginning, 54 capable of acidifying at least once in the ccurse of various tests and they obtained some mutants that acidified rhamnose from strains that usually produced only a late and inconstant acidification. Similar mutants had been obtained by action of the bacteriophage by Korobkova (1937) or on Endo's culture medium with rhamnose by Englesberg (1957).

Although this group of characters takes away from the non-fermentation of rhamnose by the plague bacillus the doctrinal strictness that the supporters of Bezsonova's medium thought that they could put into it, peptone water with rhamnose is still of some value in differentiating Malassez and Vignal's bacillus (G. Girard) provided that the technique recommended by this author to the Committee of Plague Experts in 1952 is utilized: "The favorable culture medium is inspired by the formula recommended at one time by Bezsonova, but there is an advantage in raising the proportion of peptone to 10 parts in 1,000 instead of 5 parts in 1,000 as Bezsonova recommended.

"Formula: Peptone (any type) ..... 1 g
NaCl ..... 0.5 g
Rhamnose ..... 1 g
distilled water ..... 100 ml

Adjust the pH at 6.8. Filter on an L3 bougie and distribute in 9/10 tubes in a volume of 2 to 3 ml. Add sterilely one drop of Andrade's indicator reagent in each tube (acid fuchsin: aqueous solution 0.5 parts in 100: 100 ml; NaOH N: 16 ml. Sterilize fifteen minutes at 115°C. Solution very slightly rose-colored. Keep in ampules in the refrigerator). Check the sterility and keep the tubes covered in the refrigerator in order to prevent evaporation.

"At the time of use, seed one culture flask on slanting gelose. Put in the incubator at 26° to 28°C. In case of fermentation, the medium, that is very slightly rose-colored, changes to bright red. After 24 or 48 hours the result is obtained.

"The sensitivity of the indicator is such that one may be hesitant about interpreting the result with certain plague strains. In this case we recommend a parallel test made with the same medium but without indicator. After 3 days of incubation, 4 to 5 drops of methyl red (Clark's solution with 0.1 g to 100 in alcohol diluted to 1/4). The medium becomes red if there is fermentation. It takes on a yellow tint in the opposite case.

"By way of control, an authentic strain of P. pseudotuberculosis may be seeded under the above conditions. This will provide a means of comparison as well as a rhamnose-negative strains of P. pestis."

Xylose: Its acidification in 24 hours is constant, according to unanimous opinion. The possibility of slowness is mentioned only exceptionally: 3 days (Swellengrebel and Hoesen, 1915), 5 days (Thal, 1954), or still later (Beaudette, 1940). The results were probably variable according to Hnatko and Rodin (1962). Issaly (1950-1953) determined that the acidification was identical, whether it was a question of levogyric or dextrogyric xylose.

Out of 206 strains examined by us, all of them, including Hnatko and Rodin's, acidified xylose in 24 hours.

Arabinose: Arabinose, used by a very great number of experimenters, showed itself to be acidified by almost all the strains. Nevertheless, Devignat (1954) pointed out the possibility of exceptions and strains that do not acidify arabinose are described by Urbain and Nouvel (1937), Karlsson (1945), Van Dorssen (1952), Hnatko and Rodin (1962). The possibility of late fermentation is mentioned by Moerch and Krogh-Lund (1931), Moretti 1938), Beaudette (1940), Geurden and Willems (1940), Thal (1954).

Out of 206 strains studied by us, all fermented arabinose in 24 hours, including Hnatko and Rodin's.

Russo (1940), while examining the action of nine strains, observed that dextrogyric arabinose was never acidified, only levogyric arabinose. According to Issaly (1950-53), on the other hand, L-arabinose and D-arabinose are acidified identically.

Action on hexoses.

Glucose: Except for Miller and Gladky (1927) one of whose strains produced an S-variant that did not ferment glucose, all the authors have unanimously verified its acidification by Malassez and Vignal's bacillus. The members of the Plague Commission in India (1908) insisted on the constancy of this character, as did Wu, Chung and Pollitzer (1936), etc.

There is probably always a more active acidification of glucose,

as well as of levulose, mannitol and maltose, by Malassez and Vignal's bacillus, according to Swellengrebel and Hoesen (1915). Otten (1926) based a differentiation test in peptone glucose water, 0.5 parts in 100, on this difference in activity and rapidity.

Galactose: The acidification of this substance may be considered as constant, in spite of the very rare exceptions pointed out by Lesbouyries (1934) Grancini (1939), Karlsson (1945) and Pollitzer (1954). Some strains may acidify only after 3 to 5 hours (Swellengrebel and Hoesen, 1915; Mørch and Krogh-Lund, 1931; Rosenwald and Dickinson, 1944; Karlsson, 1945).

Out of 200 strains examined by us, all fermented galactose in 24 hours.

According to Kurauchi and Nagata (1930), Malassez and Vignal's bacillus is probably restricted less in its development by the acidity produced in a liquid medium with galactose or with levulose than Yersin's bacillus.

Levulose: The acidification of levulose is a constant character, according to every author except Dungall (1931). Of 205 strains that we examined we found no exceptions to this rule.

Swellengrebel and Hoesen (1915) insisted on the constancy and the rapidity of this acidification. According to them, this last character constitutes a good argument for differentiation from Yersin's bacillus.

Let us recall that one of Gate and Billa's (1928) strains, already mentioned, did not acidify, on isolation, any other sugar except levulose.

Sorbose: The action on sorbose was studied by Christensen (1927), Swellengrebel and Hoesen (1915), Russo (1939), Macchiavello (1941) who never saw it acidified by Malassez and Vignal's bacillus. Only Tumansky (1958) considers the acidification of sorbose to be constant.

Dextrose: All the writers and especially Saisawa (1909-1913), Lerche (1927), Schütze (1927), Christensen (1927), Truche and Bauche (1929), Bishop (1932), F. Kauffmann (1933), Russo (1939), Moss and Battle (1941) are unanimous in affirming the acidification of dextrose by Malassez and Vignal's bacillus.

Mannose: The acidification of mannose is constant, according to all those who have conducted research on the action of Malassez and Vignal's bacillus on this hexose (Mørch and Krogh-Lund, 1931; Bishop, 1932; F. Kauffmann, 1933; Russo, 1939; Marthedal and Velling, 1954; Devignat, 1954). Karlsson (1945) alone points out a strain that did not ferment it.

Fucose: We found in studies written on this subject that two authors who conducted research on the action of Malassez and Vignal's bacillus on fucose, Russo (1939) with nine strains and Macchiavello (1941) with only one, observed the absence of acidification.

Action on disaccharides.

Lactose: The absence of acidification of lactose, initially observed by Pfeiffer (1889) and Lucet (1898), was confirmed by every subsequent study.

Russo (1939), Swellengrebel and Hoesen (1915) and Macchiavello (1941) alone admit the possibility of an acidification of lactose by Malassez and Vignal's bacillus. Miller and Gladky (1927) likewise claimed that it could be acidified, if not in bouillon, at least in Barsiekov's culture medium, but Kurauchi (1931), using the same medium (nutrose-lactose-litmus bouillon), observed no acidification after three weeks of observation. Knapp (1959) obtained acidification of lactose only late (4 to 5 weeks) and by four strains only. We never found it ourselves in 617 strains that we examined.

On the other hand, with Yersin's bacillus, according to Devignat (1954): "lactose often displays a slight but definite fermentation only after a rather long contact time, on the order of one to two weeks and only in the presence of phencl red as an indicator. We never saw a change in lactose culture medium with the addition of Andrade's indicator. This acidification of lactose, pointed out by Pollitzer and that Kurauchi considers as constant but slow (three weeks) and weak (pH 6.1), may be accelerated in the presence of phenol red, by seeding non-proliferative germs in a container with sugars. We observed it irregularly in strains of three varieties, but none of the pseudotuberculosis strains made lactose ferment under the same conditions. Therefore, in practice, it is necessary to consider lactose as irregularly, weakly and slowly hydrolyzable by P. pestis and as non-decomposable by Pasteurella pseudotuberculosis."

β galactosidase (O.N.P.G. reaction).

Le Minor and Ben Hamida (1962) after having demonstrated a β galactosidase in different Enterobacteriaceae, made the same investigation by way of comparison in other species and demonstrated its presence in four strains of Malassez and Vignal's bacillus at the same time as they established its absence in two strains of P. septica. We conducted the same research (1962) with Le Minor in 240 strains of Malassez and Vignal's bacillus, 72 strains of Yersin's bacillus, 19 strains of P. tularensis, 24 strains of P. septica, 1 strain of P. hemolytica and 1 of P. novicida. All the strains of Malassez and Vigna's bacillus used, regardless of their serologic type, date of isolation, animal or geographic origin, gave evidence of having a β galactosidase that produced a strongly and rapidly positive reaction. The same is true of Yersin's bacillus and P. hemolytica. On the other hand, P. tularensis and P. novicida gave evidence of lacking this enzyme, as did all of our strains of P. septica. We thought that we had found there an additional argument, if one was necessary, for differentiating P. septica from Yersin's and Malassez and Vignal's bacilli, but we are endebted to W. Frederiksen for 5 authentic strains of P. septica that acidified lactose in 24 hours and therefore, produced a fortiori a positive O.N.P.G. reaction. This differential character, therefore, does not have the value that we thought that we could attribute to it.

Maltose: Since the initial observations of Vourloud (1908), Schastny (1910) and Swellengrebel and Hoesen (1915), every writer has confirmed the acidification of maltose by

Malassez and Vignal's bacillus and Yersin's bacillus. Studies on the subject mention only two strains that do not acidify this disaccharide, Messerschmidt and Keller's (1914) and Moss and Battle's (1941) "New Orleans" strain. In fact, this last mentioned strain, that has been preserved in the laboratory since 1948, gave us a rapid fermentation of maltose when we studied it in 1961. Beaudette (1940) isolated, in a blackbird, a strain that did not acidify maltose in 48 hours at 37°, but that did acidify it after remaining another 24 hours at room temperature. After it was transplanted in a mouse, the same strain acidified maltose culture mediums in 24 hours at 37°.

Every one of the 207 strains whose behavior we examined, produced acidification in 24 to 48 hours at 37°.

The rapidity of acidification of maltose, greater with Malassez and Vignal's bacillus than with Yersin's bacillus, as Swellengrebel and Hoesen (1915) and Schastny (1910) pointed out, was utilized by the latter who suggested litmus-maltose gelose, 1 part in 100, as a differential culture medium. Himmelfarb (1927) used the same principle, but in a liquid culture medium: peptone water, 0.5 parts in 100, containing 0.5 to 1 part in 100 of maltose, incubated at 28°-30° for 48 to 72 hours after which a few drops of methyl red are added. The differences in staining are unfortunately too slight to make a sure differentiation possible (Zlatogoroff; Boncinelli and Aradas, 1933).

Sucrose: In contrast with P. septica, Malassez and Vignal's bacillus never acidifies sucrose, according to almost all writers on the subject, provided that specimens carefully checked beforehand by Fehling's reaction (Devignat, 1954) are utilized. Vourloud (1908) Swellengrebel and Hoesen (1915) alone consider this acidification to be habitual.

Some exceptions are admitted by Wu, Chung and Pollitzer (1936), Topley and Wilson (1955), Bergey (1957), Knapp (1959-1960) and Prevot (1961).

Of 10 strains isolated in a hare by Goyon (1956), one acidified sucrose.

The acidification is probably irregular, according to Urbain and Nouvel (1937), Beaudette (1940), Gaiger and Davies (1955).

Verge and colleagues (1937) isolated in a cat a strain that acidified sucrose in 48 hours and then alkalized the medium toward the tenth day. The same was true for Moss and Battle (1941) with the "New Orleans" strain, but, in our hands, this last-mentioned strain never gave us similar reactions.

Laurynowicz (1925) isolated in the urine of a little girl a strain that acidified sucrose weakly. Another one, isolated by Mathey and Siddle (1954) in a turkey, acidified sucrose, when isolated, but lost this property after several transplantings.

Klesig and colleagues (1949) described two strains as acidifying sucrose that Knapp and we, who examined them, cannot consider as authentic Malassez and Vignal's bacilli.

We observed no acidification of sucrose in 218 strains examined and we consider this character as a good argument for differentiation with P. septica.

Trehalose:

Its acidification is constant for all writers on the subject. Seal (1951) alone points out two strains (P.R. (I)) and (P. R. (III)) that do not acidify trehalose. Wolff (1956) puts the fermentation of trehalose on the same level as the investigation of mobility for differentiating Malassez and Vignal's bacillus and Yersin's bacillus, a belief that cannot be retained. Acidification of trehalose by Yersin's bacillus was variable for Pollitzer (1954) and constant for Devignat (1954).

Melibiose: Melibiose is acidified by Malassez and Vignal's bacillus, according to Russo (1939), Prado (1940), Macchiavello (1941), Pollitzer (1954), Winkle (1955) and Knapp (1959-1960). Knapp who admits the possibility of exceptions, contrasts this acidification of Melibiose to the absence of acidification by P. septica and by Yersin's bacillus (1960). Russo and Prado likewise consider that the fermentation of melibiose is peculiar to Malassez and Vignal's bacillus and is as valuable as the acidification of rhamnose in differentiating it from Yersin's bacillus. Devignat (1954) confirmed the rapid acidification (24 hours) of melibiose by three strains of Malassez and Vignal's bacillus "whereas only medieval strains of P. pestis acidified it toward the 70th day. This glucide, therefore, could appear with rhamnose among the substances that make a rapid differentiation possible, except where the medieval variety of P. pestis is present."

Cellobiose: Not acidified, according to Devignat (1954) and Christianssen (1918), cellobiose is probably acidified, according to Russo (1939), but with exceptions. Macchiavello (1941) described a strain that acidified cellobiose slowly.

Action on trisaccharides.

Melezitose: Melezitose is never acidified either by Malassez and Vignal's bacillus (Christensen, 1927); Mørch and Krogh-Lund, 1931; F. Kauffmann, 1933; Russo, 1939; Savino and Anchezar, 1939; Beaudette, 1940; Macchiavello, 1941; Tumansky, 1958; Knapp, 1959), or by Yersin's bacillus (Devignat, 1954).

Melitose:

Melitose is not usually acidified, although the possibility of exceptions is admitted by Murray-Pullar (1932), Gourden and Willems (1940), Seal (1951), Devignat (1954), Pollitzer (1954), Klimova (1956), Tumansky (1958). According to Knapp (1960), its behavior is probably variable.

Action on polymerized glucides.

Amidon: Amidon is acidified, according to Mørch and Krogh-Lund (1931), Russo (1939), Savino and Anchezar (1939), Macchiavello (1941). Devignat (1954) confirmed this acidification to which, however, there are exceptions, such as Beaudette's (1940) strain, Gourden and Willems's (1940) strains, a strain isolated in a cat by Verge and colleagues (1937), the strain isolated in a toucan by Urbain and Nouvel (1937).

According to Kauffmann (1933) and Kodrnja (1933) the action

probably varies with the strains.

In 150 strains of Malassez and Vignal's bacillus that we examined, acidification of amidon was always obtained in 24 hours.

Inulin: Almost every author (Galli-Valerio, 1902-1903; Kakehi, 1916; Roemisch, 1921; Christensen, 1927; Schütze, 1928; Mørch and Krogh-Lund, 1931. F. Kauffmann, 1933; Uriarte and Villazon, 1935; Verge and colleagues, 1937; Savino and Anchezar, 1939; Seal, 1951; Klimova, 1956 consider inulin as consistently not acidified by Malassez and Vignal's bacillus.

Some exceptions are admitted by certain writers (Pollitzer, 1954; Winkle, 1955). According to Tumansky (1958), the results are variable, but generally there is no acidification.

Nevertheless, Swellengrebel and Hoesen (1915), Russo (1939) described strains that acidify inulin and Mendonca Machado and Transmontano Pelouro (1943) found five such strains out of ten strains studied by them. Knapp, after having considered, in an earlier study (1959), the acidification of inulin to be exceptional, gives it as almost constant in a later publication (1960).

According to Devig at (1954) (1961), divergencies in these results are due to the choice of the indicator: "The five strains of Pasteurella pseudotuberculosis that I studied gave a weak acidification that is revealed only with indicators as sensitive as phenol red or bromothymol blue. Writers who state that there is no fermentation of inulin worked, in my opinion, with indicators like litmus or Andrade's indicator with acid fuchsin that are not very sensitive. With phenol red, that turns yellow at pH $\pm$ 6.9, and bromothymol blue, that turns yello at pH $\pm$ 6, acidification is constantly detectable, at least for the five strains of Pasteurella pseudotuberculosis on which we experimented."

Glycogen: Its acidification was obtained by Mørch and Krogh-Lund (1931) with about thirty strains, and Tumansky (1938) considers it as constant.

Russo (1939), Macchiavello (1941), Pollitzer (1954), Winkle (1955) believe that Malassez and Vignal's bacillus does not acidify glycogen, with very rare exceptions. The results are probably variable, according to F. Kauffmann (1933).

For Prado (1940) constant acidification by Yersin's bacillus and non-acidification by Malassez and Vignal's bacillus are the rule. In fact, irregularities in the behavior of Malassez and Vignal's bacillus and Yersin's bacillus (Matumoto, 1949) do not enable us to confirm this rule.

Dextrin: Opinions differ on the action of Malassez and Vignal's bacillus on this body: there is probably no fermentation, according to Saisawa (1913), Baumann (1927), F. Kauffmann (1933), Lesbouyries (1934), Barsini (1935), Uriarte and Villazon (1935), Verge and colleagues (1937), Russo (1939), Geurden and Willems (1940), Rosenwald and Dickinson (1944), Karlsson (1945), Marthedal and Welling (1954), Van Dorssen (1955), Topley and Wilson (1955).

There is probably a slow, weak acidification, according to Mørch and Krogh-Lund (1931), Beaudette (1940), Macciavello (1941), Devignat (1954). Acidification is probably constant, according to MacConkey (1908), Swellengrebel and Hoesen (1915), Lerche (1927), Christensen (1927), Kurauchi (1931), Savino and Anchezar (1939).

Boncinelli (1932), who considered this fermentation as constant, suggested a synthetic dextrin culture medium at pH 6 and including, as indicator, bromothymol blue. Only Malassez and Vignal's bacillus probably produces a change to blue-green.

Knapp, after he had originally (1955) given acidifcation as the rule with the possibility of exceptions, changed his mind on this point (1960) and considers the action to be variable, sharing thus in the opinion of Seal (1951), Pollitzer (1954) and K. F. Meyer (1958).

We believe the acidification of dextrin to be constant, provided that a peptone suitable for this test is used. 170 strains did not acidify in ten days in the presence of PTV peptone, whereas they did so in 24 hours in the presence of IBF peptone.

Action on polyalcohols.

Glycerol: Dujardin-Beaumetz and Simard (1925), when they were making a comparatively study of their plague strains with a strain of Malassez and Vignal's bacillus, noted, contrarily to Vourloud, that this strain acidified glycerin culture mediums. Colas-Belcour (1926) verified the reality of this fermentation and proposed glycerin-litmus gelose as a differential culture medium. Bezsonova and Knovalova (1927) confirmed Colas-Belcour's results in so far as action of Malassez and Vignal's bacillus on glycerol is concerned, but took away quite a bit of the diagnostic importance of this action by demonstrating the existence of a glycerol-positive variety of Yersin's bacillus. G. Girard (1928) confirmed Colas-Belcour's observations on the behavior of Malassez and Vignal's bacillus, but Kurauchi (1930), then Kowasima (1934) and Matumoto (1949) demonstrated in turn the existence of two varieties of Yersin's bacilli of which Kurauchi was the first to point out the geographic distribution.

Although the fermentation of glycerol is no longer a formal proof of differentiation from the plague bacillus, it still is a constant character of Malassez and Vignal's bacillus [See Note 1]. Of 617 strains that we examined we never found any that did not comply with this rule. In a bibliography that is especially rich on this point, Castellani (1938-1939) and Tumansky (1958) are the only ones who point out the possibility of exceptions [See Note 2]. Castellani, who admits the possibility of non-fermentation of glycerol by certain strains, similar to rhamnose for certain other strains, suggested a differential culture medium including these two bodies.

([Note 1:] With an important reservation, in view of Brunet's (1952) production of strains that lost all effect on glycerol after action of the bacteriophage.)

([Note 2:] Marracini (1953) described a strain that does not acidify glycerol, but whose other characters (non-fermentation of rhamnose, non-reduction of nitrates) make one at least suspect the authenticity.)

The possibility of slowness in acidification of glycerin culture mediums has been pointed out: three days (Swellengrebel and Hoesen, 1915; Mathey and Siddle, 1954), five days (Piechaud, 1952), eight days (Ceurden and Willems, 1940; Lesbouyries, 1941).

The choice of the culture medium assumes a great importance in the study of the action on glycerol. A number of authors have noted the absence of acidification by Malassez and Vignal's bacillus

in Stern's medium (F. Kauffmann, 1933; Savino and Anchezar, 1939) or its slowness (Lesbouyries, 1941). Therefore, the technique recommended by Girard to the World Health Organization (1953) will be used: "Distribute in 16 to 18 mm test tubes 6 ml of peptone gelose (20 parts in 1,000 of a peptone such as bactopeptone, tryptose, casein, Oclaf) salted 5 parts in 1,000 and filtered, and add six drops of neutral glycerol per tube. Adjust the pH at 6.8, sterilize at 115°C for 20 minutes. Let it cool to 50° C and add two drops of Andrade's reagent in each tube. Shake slowly and let the gelose assume a slanted position.

"Seed rather generously on the basis of a culture of slant gelose and incubate at 28°-32°. Already after 24 hours, often after 48 hours and always after 5 days, the result is obtained. The more or less intense red tint of the culture medium indicates that there has been acid fermentation of the glycerol."

One of the two complex differential mediums suggested by Devignat (1953), (culture medium I), is based on the fermentation of glycerol, of rhamnos, and on the reduction of nitrates.

Mannitol: Although Tumansky (1958), even though he considers this alcohol as usually acidified by Malassez and Vignal's bacillus, gives this character, nevertheless, as variable, all the writers and we consider this fermentation as constant. We verified it in 600 strains.

We have found, in writing on the subject, only two strains described as not acidifying mannitol: one of them isolated in a duck by Krauss and Hensel (1961), the other one isolated in man by Moss and Battle (1941). Nevertheless, we saw the second of these two strains acidify mannitol in 24 hours.

The two strains described by Hässig and colleagues did not produce acidification, but we have already expressed reservations on their authenticity.

Swellengrebel and Hoesen (1915), who believed that mannitol is acidified more actively by Malassez and Vignal's bacillus than by Yersin's bacillus, offered this fermentation as a criterium of differentiation.

Adonitol: Adonitol is probably not acidified, according to Christensen (1927), Savino (1939), Geurden and Willems (1940), Marthedal (1954), Devignat (1954), Pollitzer (1954). It is probably constantly acidified, according to Vourloud (1908), Galli-Valerio (1902-1903), Kakehi (1916), Schütze (1928), Truche and Bauche (1930), Kurauchi (1931), Bishop (1932), Uriarte and Villazon (1941), Macchiavello (1941), Lesbouyries (1941), Mendonca (1943), Cecarelli (1950), Winkle (1955).

The action is variable, according to Thal (1954).

For Tumansky (1958) there probably is no acidification, generally, whereas Knapp (1959) considers acidification as the rule with some exceptions.

This acidification, like the acidification of salicin, is constant for Haupt (1928) and is sufficient, according to him, for differentiation with Salmonella gallinarum.

A frequent slowness in acidification was noted by Bishop (1932) with six strains of Malassez and Vignal's bacillus and Kakehi (1916) demonstrated that the divergencies were due to this slowness

in acidification: "every strain of this organism, without exception, forms acid in the space of a certain length of time with considerable individual differences in the rapidity of the action. Such action was not noted by MacConkey (1905), Petrue and Macalister (1911)and Saisawa (1913), due probably to the fact that the observation was not pursued for a sufficiently long time." Kakehi demonstrated, besides, that "delays in the change that begins after seven to ten days and does not reach its maximum until after 21 and 24 days may be shortened by transplanting in an adonitol medium. The maximum is reached in 10 to 14 days by the first sub-culture and in 5 to 7 days by the second one. However, some strains may produce only a very weak acidification that will not be visible until close to the 28th day."

One of Beaudette's (1940) strains acidified adonitol slowly only after being transplanted in a mouse.

According to our own results, one strain out of two does not ferment adonitol, regardless of the length of observation time.

Sorbitol: It is not acidified, according to Lerche (1927), Beck (1928), Schütze (1928), Moerch and Krogh-Lund (1931), Russo (1939), Thal (1954), Devignat (1954), Tumansky (1958), etc. According to Brigham and Rettger (1935), this character, that is common to Malassez and Vignal's bacillus and to Yersin's bacillus, contrasts these germs with P. septica, an opinion confirmed by Knapp (1959-1960) who admits, however, the possibility of exceptions in so far as Malassez and Vignal's bacillus is concerned.

The acidification is variable, according to Pollitzer (1954), K. F. Meyer (1955), Hnatko and Rodin (1962). It is probably weak, according to Macchiavello (1941) and constant for Galli-Valerio (1902), Savino and Anchezar (1939), Geurden and Willems (1940).

Strains that acidify sorbitol have also been described by Topping and colleagues (1938), Schaffer (1939),Cecarelli (1950). Strains of Hässig and colleagues (1949), Terni and Kircheis (1950) cannot be retained.

According to our experience, the action of Malassez and Vignal's bacillus on sorbitol is variable. Although three-fourths of the strains produced no acidification, some of them, among the rest, are capable of producing a commencement of change and certain ones, more rare, acidify definitely.

Dulcitol: Its non-acidification is almost constant. Pollitzer (1954), Winkle (1955), Tumansky (1958), Knapp (1959), mention the possibility of exceptions. We are familiar only with the ones pointed out by Vourloud (1908), Swellengrebel and Hoesen (1915), Roemisch (1919), Schäffer (1939) and Barsini (1935) and we ourselves did not find any acidification in 300 strains examined.

Zlatogoroff reports on a strain whose R-variant, alone, acidified oulcitol. Roemisch (1921) observed the opposite phenomenon.

Inositol: It is never acidified, according to all the writers, and we did not find any exceptions in 200 strains that we examined.

Erythrol: It is never acidified, according to Schütze (1928), March and Krogh-Lund (1931), Kauffmann (1933), Russo (1939),

Devignat (1954), Pollitzer (1954), Klimova (1956), Knapp (1959-1960), etc. Only Galli-Valerio (1902-1903) and Tumansky (1958) mention the possibility of exceptions.

Arabitol: Savino and Anchezar (1939) consider it as not not acidified, as does Russo (1939) who points out, however, the possibility of exceptions. One strain isolated in man by Macchiavello (1941) acidified arabitol weakly and slowly.

Quercitol: The action of Malassez and Vignal's bacillus on quercitol, not acidified by Yersin's bacillus, according to d'Aunoy (1923), and acidified, according to Tumansky (1958), was not studied.

Action on glucosides.

Esculin: Russo (1939) was the first to mention the acidification of esculin by Malassez and Vignal's bacillus, a result that was confirmed by Macchiavello (1941), Jamiesson and Soltys (1946), Tumansky (1958). Mendonca Machado and Transmontano Pelouro (1943) were the only ones who did not observe this acidification that Parnas (1961) confirmed with 45 strains studied in bouillon with 1 part in 1,000 of esculin. In an eralirer study (1961) we obtained acidification in less than 24 hours with 318 strains out of 327 on gelose that contained 1 part in 1,000 of esculin. Only two strains induced a late darkening (13 and 15 days), whereas another strain acidified esculin only after being transplanted on a rabbit or by action of the bacteriophage. Only six remained completely negative. In the present study, of 617 strains that we examined we found only 13 that did not acidify esculin.

The darkening of the seeding injection is usually appreciable during the very first hours of cultivation. If it occurs later, it always appears first at 28°.

Esculin is also acidified by Yersin's bacillus in less than 24 hours and it never is by P. septica.

Amygdalin: It is not acidified, according to Christensen (1927), Schütze (1928), Mørch and Krogh-Lund (1930), F. Kauffmann (1933), Russo (1939), Devignat (1954), Klimova (1956), K. F. Meyer (1958), Tumansky (1959). The possibility of exceptions is pointed out by Vourloud (1906) and Knapp (1959). According to Pollitzer (1954), on the contrary, acidification is the rule.

Miravet the Issaly (1950-1953), while making a parallel study of the action on amygdalin, salicin, arbutin of 11 strains of Malassez and Vignal's bacillus, 11 strains of Yersin's bacillus and 48 strains of P. septica, did not see any of these strains produce a change in a gelose culture medium containing 1 part in 100 of amygdalin.

Arbutin: Acidification of arbutin by Malassez and Vignal's bacillus was noted by Christensen (1927), Schütze (1928), Barsini (1935), Russo (1939), Macchiavello (1941). Miravet de Issaly confirmed that Malassez and Vignal's bacillus, like Yersin's bacillus, acidified arbutin but P. septica did not. According to these writers, its fermentation is probably on a par with the fermentation of salicin.

Salicin: According to the general opinion, salicin is acidified almost constantly. Although Knapp and Masshof (1954), in an earlier study, concluded that there was no acidification, Knapp (1959-1960) reversed himself on this point and considers the acidification of salicin as constant, with very few exceptions. Some exceptions were also pointed out by Christensen (1927), Haupt (1928), Pollitzer (1954), Winkle (1955). The possibility of slowness in acidification was pointed out (Christensen, 1927); Bishop, 1932; Mørch and Krogh-Lund, 1931; Gourden and Willems, 1940; Rosenwald and Dickinson, 1944; Thal, 1954; Hnatko and Rodin (1962).

The action is probably variable, according to Kodrnja (1933), Stephan (1941), Marthedal and Welling (1954).

According to Snyder and Vogel (1943), the "Spokane" strain produces acidification in 24 hours, and then alkalization on the third day.

Haupt (1928), although he admitted the possibility of exceptions, saw in the acidification of salicin by Malassez and Vignal's bacillus a good element of differentiation with Samonella gallinarum and Kurauchi and Nagata (1950) contrasted fermentation by Malassez and Vignal's bacillus and by Yersin's bacillus with the absence of acidification by P. septica, a result confirmed by Issaly (1950-1953) who obtained acidification in 24 hours of a gelose culture medium, 1 part in 100 of salicin, by Yersin's bacillus and by Malassez and Vignal's bacillus, whereas one single strain out of 48 of P. septica gave him the same result.

The choice of the peptone is especially important for investigating the acidification of salicin. 142 strains examined by us did not give us any acidification after three weeks of observation in peptone water containing 2 parts in 100 of salicin when we used FTV peptone, whereas the change was constant in 24 hours with IBF peptone.

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Various reactions must be considered in relation to the acidification of carbohydrate substances:

b. Production of acetylmethylcarbinol.

Malassez and Vignal's bacillus and Yersin's bacillus do not produce acetylmethylcarbinol (negative Voges-Proskauer reaction).

Test with methyl red: Malassez and Vignal's bacillus and Yersin's bacillus produce in a Clark and Lubs culture medium an evident acidity that is disclosed by testing with methyl red. Mathey and Siddle (1954) alone pointed out a strain that gives a positive reaction at the time of its isolation and then a negative one with the subsequent sub-cultures.

c. Action on sodium citrate.

M. Piechaud (1952), while studying a strain recently isolated in a man, observed that it did not develop on a Simmons's culture medium. According to Thal (1954) and Devignat (1954), Malassez and Vignal's bacillus and Yersin's bacillus are incapable of utilising sodium citrate as the sole source of carbon. Knapp (1959) seeded the same medium with 14 strains of Malassez and Vignal's bacillus of

types I to V and observed that, although there was no culture at 37°, three strains yielded a minimum culture at 22° in 10 to 21 days.

We studied (1961) the behavior of 327 strains of Malassez and Vignal's bacillus at 18°, 28° and 37° on Simmons's medium, Christensen's medium and in citrate bouillon, 1 part in 100, with the following results:

No strain develops at 37° on Simmons's medium. On the other hand, at 18° most of the strains of types I, II, III and V produced a very poor culture starting with the tenth day, on the average, with, more slowly, a definite change of the medium. This change appears, generally, in two to three weeks and even later, toward the 78th day.

This change is due to mutants that produce the appearance of rare white colonies contrasting with the yellowish appearance of the rest of the culture. The number of these colonies varies according to the strains and often, when various tests were made, with one same strain, due no doubt to the more or less large amount of inoculum.

Transplanting these colonies on a Simmons citrate culture medium shortens progressively the time of change down to 48 hours, as Marneffe had already observed in 1959. These mutants, nevertheless, remain incapable of growing at 37° on Simmons's culture medium.

At 28° one strain out of three, approximately, does not develop or produces only a very meager culture, without ever causing a change in the medium. Those that develop at this temperature always produce a change still later than at 18°.

The addition of two drops of glucose, 30 parts in 100, per tube, according to D. Piechaud's (1962) technique, shortens very appreciably the time of change that then usually occurs in less than one week and, most frequently, in 48 hours. But even under these conditions it is still impossible to obtain a culture at 37°.

The behavior of strains of type IV is appreciably different from the behavior of strains of other types. Although these strains do not develop at 37° any more so than the others at 18° and at 28°, a change in the medium occurs in much shorter periods of time than the ones observed with most of the type I, II, III and V strains. Although this change did not appear until the 15th day in certain tests, we obtained it in most cases between two and four days.

On Christensen's culture medium the results are identical, with the same precocity of change by type IV strains.

In citrate bouillon, 1 part in 100, most of the strains of Malassez and Vignal's bacillus acidified the citrate in 15 days, but only at 18°.

Yersin's bacillus and P. septica do not modify these three mediums.

d. Fermentation of tartrates and mucate.

Neither D-tartrate (Kauffmann, 1933; Stephan, 1941; Cecarelli, 1950; Thal, 1954; J. Schmidt, 1959; Mollaret, 1961), nor L- and I-tartaric acids (Mollaret, 1961), nor sodium mucate (Thal, 1954), are acidified by Malassez and Vignal's or Yersin's bacilli.

Fermentation of sodium malonate. In Leifson's sodium malonate bouillon none of the 327 strains of Malassez and Vignal's bacillus of types I, II, III and V that we studied (1961) acidified malonate, regardless of the incubation temperature. On the other hand, the

three strains of type IV that we had at the time produced a change of the medium in 48 hours at 28° and in 4 to 7 days at 18°, whereas there was no modification at 37°.

Two other strains of type IV that we examined subsequently yielded the same results. On the other hand, a sixth strain of the same type, isolated by J. Bouton and E. G. Hall using the stools of a child suffering from meseteric adenitis did not acidify malonate. This fermentation, although it seems indeed to be an exclusive attribute of type IV of Malassez and Vignal's bacillus, is not, however, a constant character of it.

The absence of acidification of malonate by Malassez and Vignal's bacillus was confirmed by Steel and Midgley (1962).

3. Metabolism of nitrogenous substances.

a. Proteolytic power.

Pfeiffer (1889) was the first to observe that Malassez and Vignal's bacillus is lacking in any proteolytic power. Neither plain gelatin, nor Kohn's gelatin, nor coagulated serum or egg albumin is acidified by it. There is unanimous agreement on this point and no strain in our collection was an exception to this rule, regardless of the incubation temperatures and the length of observation.

b. Decomposition of urea.

The decomposition of urea by Malassez and Vignal's bacillus is an important character both for its constancy and for the fact that there are no strains of Yersin's bacillus that show this same property.

Urease in Malassez and Vignal's bacillus was demonstrated for the first time in 1950 in the general microbiology laboratory of the Pasteur Institute by J. Gaillard whose work was not published. In the same year, Fauconnier and Chevalier (1950) made known the identical results obtained by them in G. Girard's laboratory. These results were confirmed by Issaly (1950-1953) and then by Thal (1954) with 186 strains and by Thal and Chen (1954) with 204 strains of Malassez and Vignal's bacillus and 50 strains of Yersin's bacillus.

In 1961 we examined the behavior of 327 strains of Malassez and Vignal's bacillus at 18°, 27° and 37° in the following culture mediums: Ferguson's medium, Roland-Bourbon-Szturm's urea-indole medium, Christensen's urea medium, and placenta-urea bouillon recommended by Sohier for proving the presence of urease in certain Corynebacterium with the following formula:

(Sohrab 1947) placenta bouillon	100 ml
Urea solution, 50 parts in 100	5 ml
Creson red solution, 0.4 parts in 100	5 ml

In a urea-indole medium and in Ferguson's medium, we observed a change in less than four hours, as Fauconnier had demonstrated, most often within an hour, some times in fifteen minutes or even almost immediately. In these time-periods the temperature did not modify the rapidity of the reaction. On the other hand, with some strains that give a late reaction the change always appears first at 37°. In these cases the time required for the change according to temperature is usually definite: for example, change in six hours at 37° and in twenty hours at 18°.

On Christensen's medium change appears toward the 20th hour, as Thal and Chen observed. Temperature does not modify this period of time.

In placenta-urea bouillon, the change starts toward the sixth hour at 37° and always later at 28° and especially at 18°. This last-mentioned culture medium gave us more consistently positive results than the other three. Thus, strain 35-II ("pheasant" strain) that Fauconnier, in 1950, had found to be negative in a urea-indole medium and in Ferguson's culture medium and that had given the same results to Devignat in 1954, also showed up as negative to us on

those two mediums as well as on Christensen's culture medium, but it showed itself as positive in a placenta medium (the controls to which urea had naturally not been added did not change).

On the other hand, three recently isolated strains (230-I, 231-I and 232-I) that we found to be positive late in a urea-indole culture medium, Ferguson's medium and Christensen's medium (more than 24 hours and, for one of them, more than 48 hours), showed up, on the contrary, from the time of isolation as positive in placenta bouillon (successive transplantings of these strains on gelose progressively reduced for two of them the time required for change in a urea-indole medium and in Ferguson's medium to six hours beginning with the third transplanting and to four hours beginning with the eihth. This explains, without doubt, why we found strain 25-II, that in 1950 Fauconnier had found to be negative on these same mediums, to be positive in four hours on a urea-indole medium and on Ferguson's medium).

The decomposition of urea by Malassez and Vignal's bacillus is, therefore, a constant character in the 617 strains of this germ that we examined. Although only 4 produced a late change on a urea-indole culture medium and on Ferguson's medium and only one remained definitely negative on these same mediums, all of them appeared to be capable of decomposing urea in placenta bouillon.

On the four mediums used, Yersin's bacillus and P. septica always showed that they were lacking in urease.

Up until the present time no strain of Malassez and Vignal's bacillus has been found that is not capable of decomposing urea [See Note 1], whereas every strain of Yersin's bacillus studied by a great number of writers has always been demonstrated as incapable of doing so [See Note 2]. The value of this test is increased all the more by the fact that, although Brunet (1952) succeeded in making Malassez and Vignal's bacillus lose, by action of bacteriophage, two of its most important characters, mobility and the capacity for acidifying glycerol, its behavior in the presence of urea did not, on the other hand, undergo any modification.

([Note 1:] The strains published by Hässig and colleagues (1949) as lacking in urease have too many aberrant characters to be retained, and, although Ul'Janova (1961) mentions three strains of Malassez and Vignal's bacillus lacking in urease, we have received from Madame Boulanova Juscenko, who studied these strains in the Gamaleia Institute, assurance that they really decomposed urea in 24 hours.)

([Note 2:] The strain isolated by Raynes (1950), so peculiar in several respects and whose ureasic activity Fauconnier (1950) verified, will not be retained. On the other hand, G. Girard (1953) pointed out a strain of Yersin's bacillus capable of decomposing urea. We took up the study of this strain, isolated in man in Kenya by Heisch. It produces, in effect, a change of the urea-indole medium in three days and of Christensen's medium in 8 days, but it has, however, no effect on the placenta-urea bouillon.)

The establishment of urease constitutes, therefore, one of the best, and even, in our opinion, the surest criteria of differentiation. Although the use of the placenta-urea culture medium turned out to be indispensable for several rare strains, in current practice Ferguson's medium, the urea-indole medium and Christensen's urea medium or the complex urea-inulin-mobility medium of Devignat (1953-1954) make a definite diagnosis easily possible.

c. Production of catabolites.

Indole: Ever since the first observation that was made by Lucet (1898), the absence of the formation of indole by Malassez and Vignal's bacillus has been confirmed unanimously.

Zeiss (1914) described a strain that produced indole but whose other characters left great doubts on its real identity. Only two writers obtained traces of indole in peptone water (Murray-Pullar, 1932 and Sautu Riestra, 1929), the latter after eight days of cultivation.

Of more than 600 strains examined by us none produced indole in peptone water or in a urea-indole medium after ten days of incubation at different temperatures. This character that may be considered as absolute in Malassez and Vignal's bacillus and in Yersin's bacillus is compared with the constant production of indole by P. septica.

Ammonia: The production of ammonia by Malassez and Vignal's bacillus is constant (Wilson and Miles, 1955; Bergey, 1957; J. Dumas, 1958, etc.). It is probably less strong than the production of ammonia by Yersin's bacillus, according to Devignat (1954).

Nitrous acid: The reduction of nitrates into nitrites has often been confused with the production of nitrous acid at the expense of the proteins in a culture medium strictly lacking in nitrates, since both phenomena are controlled by the same reaction (Griess's reaction). The Manual of Methods for Pure Culture Study of Bacteria (1942) and Devignat (1952) insisted rightfully on the need for distinguishing these two actions whose confusion accounts for the discrepancies in the results of some authors who adhered to the differentiation of Malassez and Vignal's bacillus and Yersin's bacillus by establishing one or the other of these actions.

Thus Fusco (1927-1932) and Micheletti (1932), after having concluded that "every culture that does not give a positive response to Griess's reaction is not a plague culture", remove, nevertheless, all value from this reaction for purpose of differentiating with Malassez and Vignal's bacillus, because they also obtained a positive reaction with a strain of this bacillus (strain "PG 33" of the Lister Institute). Gore (1930), on the other hand, although he confirmed the presence of nitrite in plague cultures in peptone water without the addition of nitrate, did not observe any in cultures of Malassez and Vignal's bacillus in the same culture medium.

Knovalova (1930) demonstrated, while examining 10 strains of this bacillus, that, although they all reduced nitrates, only two produced nitrites in mediums lacking in nitrate, whereas out of 146 plague strains only 9 reduced nitrates and 6 gave a positive reaction in bouillon without nitrate. According to these conclusions, and according to Wu, Chung and Pollitzer (1936), distinction between the two germs is probably not possible with Griess's reaction.

Petragnani (1937), has a different iopinion. According to him, Yersin's bacillus, alone, probably produces nitrous acid in liver bouillon, but Girard (1940), after having obtained a "very slightly positive" reaction with the "Sydney Rowland" strain of Malassez and Vignal's bacillus with 103 strains of Yersin's bacillus, variable results with four strains and negative with nine

others, removes the absolute character from the reaction recommended by Petragnani.

These divergencies are now explained to us as much by the fact that the three varieties, medieval, oriental and ancient, of Yersin's bacillus do not have the same aptitude for reducing nitrates into nitrites or for producing nitrous acid in mediums lacking in nitrate, as they are especially explained by the possible confusion of these two phenomena.

The production of nitrous acid by the oriental and ancient varieties of Yersin's bacillus and its non-production by the medieval variety were demonstrated by Khovalova (1930), Devignat (1951), Baltazard and Aslani (1932), Devignat and Chevalier (1952), Devignat (1954).

The production of nitrous acid by Malassez and Vignal's bacillus is commented on as follows by Devignat (1954): "This production of nitrous acid does not seem to be attributable to the decomposition of peptones, in the chemical sense of the word. The few experiments that we were able to perform (1952) would seem to indicate that the extensive degradation of proteins, for example by papain, makes the substrate unfavorable, whereas the large proteinic molecules, such as the ones contained in macerated meat, are very favorable to the appearance of the phenomenon whose process must differ logically from the one that intervenes in the reduction of nitrates.

The five pseudotuberculosis strains in our possession did not give any Griess reaction in nutrient bouillon. Nevertheless, they denatured the protein or proteins involved in the reaction. In fact, while we were studying our three negative medieval strains, M.38, M.39 and M.40, on the one hand, and the five negative pseudotuberculosis strains on the other hand, we observed the following:

A sample of a four-day old culture in bouillon at 30°C of medieval strains and of pseudotuberculosis strains did not produce Griess's reaction, as had been predicted. We put all the tubes of bouillon in a bain-marie for ten minutes, in order to kill the microbes with heat. After cooling, the dead microbes are deposited in the bottom of the tube, leaving a clear liquid floating on top. We seeded in this bouillon, by means of proliferative germs, a strain of Alcaligenes faecalis, a germ that possesses the property of inducing the appearance of the NO_2 ion in plain bouillon (Henriques, 1942). After four days of this secondary cultivation, which becomes very abundant, we added Griess's indicator. We observed that the reaction was positive in the tubes where the M strains formed the primary culture and that it was negative in the five tubes in which the PST strains had been cultivated beforehand. The events transpired, therefore, as if the medieval variety of plague strains did not alter the nitrigenous protein, that remained sensitive to Alcaligenes faecalis, while the pseudotuberculosis strains denatured this same protein without causing the nitrous ion to appear or by extending the disintegration to a more advanced stage.

"In summary, the antiqua and oriental varieties of P. pestis strains nitrify certain proteins and the medieval variety leave them intact. On the other hand, all the strains of P. pseudotuberculosis denatured these same proteins, some of them, according to Girard (1940), by producing the nitrous ion, detectable by means of Griess's indicator, and others, including our five, by decomposing them in a different manner."

After we had seeded 533 strains of Malassez and Vignal's bacillus in the same culture medium as the one used to investigate the reduction of nitrates (macerated meat and peptic hydrolysate of pork belly), but without the addition of nitrate, 301 of them gave, on the third day of incubation, a strongly positive Griess reaction, whereas of the remaining 132, that were re-examined after five more days of incubation, 55 gave a definite rose coloration and 177 a completely negative response.

In the presence of PTC peptone, 518 strains out of 533 this time gave a definitely positive Griess reaction on the third day.

d. Examination for sulfuretted hydrogen.

Although, according to Haupt (1928), Schütze (1928), Gore (1930), F. Kauffmann (1933), Dujardin-Beaumetz (1934), Wu, Chung, and Pollitzer (1936), Pollitzer (1954), Thal (1954), Wildfuhr (1959), Ul'Janova (1961), etc., Malassez and Vignal's bacillus does not produce sulfuretted hydrogen, a number of writers are of a contrary opinion: Savino and Anchezar (1933), Russo (1939), Macciavello (1941), Moss and Battle (1941), Rosenwald and Dickinson (1944), Cecarelli (1950), Van Dorssen (1951), Uriarte and Villazon (1953), Gaiger and Davies (1955), Bergey (1957).

The production of hydrogen sulfide is probably variable, according to Dungall (1931) and Topley (1955); it is zero or very weak according to Knapp (1959-1960), who investigated it in Kligler's medium and on SIM Difco agar.

The differences in the techniques utilized partially account for these divergencies. Thus Grancini (1939) noted that the same strains that produced a darkening in gelose with sodium thiosulfate, in cystine gelosé, in gelose with sodium sulfate, did not produce any in gelose with potassium sulfate. According to Devignat (1954) "on gelose with lead (lead acetate Difco agar), Malassez and Vignal's bacillus darkened the medium slightly along the seeding injection whereas it is not possible to detect the slightest darkening in Kligler's culture medium. Iron, therefore, would not be suitable for use as an indicator in this study of Malassez and Vignal's bacillus."

Of 400 strains examined we never observed any darkening regardless of the incubation temperature and the length of observation, either in Kligler's medium, in Christensen's medium modified with ferric-ammoniacal citrate, in gelose medium 4 parts in 1,000 with the addition of 1 part in 1,000 of sodium hyposulfite and two drops of a solution with 1/10 of lead subacetate per tube of 10 ml of medium, or on strips of acetate paper or lead subacetate paper inserted in tubes of culture on liver gelose. Although it is constant, starting with the 5th to 7th day in gelose with lead subacetate, to observe a browning alongthe seeding injection, there is never any real darkening. Therefore, we consider that under these conditions Malassez and Vignal's bacillus does not produce hydrogen sulfide.

Rosenwald and Dickinson (1944) isolated, at the time of an epizootic in turkeys, several strains some of which gave, on isolation, some traces of hydrogen sulfide, although one did not produce any until it had been transplanted on a guinea-pig. We transplanted a certain number of our strains in a guinea-pig without making the same observation.

e. Examination for lysine decarboxylase, tryptophan deaminase and the transformation of phenylalanine in phenyl-pyruvic acid.

These reactions are negative at 18°, 27° and 37° with every strain of Malassez and Vignal's bacillus, Yersin's bacillus and P. septica (Mollaret, 1961).

Henriksen and Jyssum (1961), Steel and Midgley (1962) confirmed the absence of phenylalanine deaminase in the Pasteurella bacilli.

The establishment of an ornithine decarboxylase in P. septica is probably a supplementary differentiation criterium with Malassez and Vignal's and Yersin's bacilli, according to Steel and Midgley (1962).

f. Behavior in milk.

Malassez and Vignal's bacillus and Yersin's bacillus grow easily in milk that is never coagulated by them (Pfeiffer, 1889; Delbanco, 1896; Lucet, 1898-1899; Klein, 1899-1900; Zwick, 1908; Schütze, 1928; Dujardin-Beaumetz, 1934, etc.). Only Galli-Valerio (1903) contrasted the coagulation of milk by Malassez and Vignal's bacillus with the absence of coagulation by Yersin's bacillus, an assertion criticized by Lehmann and Leumann (1907), Vourloud (1908), etc. The few strains described as capable of producing a slow coagulation (Cagnetto, 1905) were certainly not authentic Malassez and Vignal's bacilli.

The progressive alkalization of litmus milk was mentioned by Schütze (1928), Savino and Anchezar (1939), Mathey and Siddle (1954), etc. According to MacConkey (1908) and Kakehi (1910), it appears toward the 3rd to 4th day at 37° and reaches its maximum on the 5th day. According to Reis and Nobrega (1936), it took longer (5 to 7 days.) It is slow and weak for Topley (1955) but constant for MacConkey (1908) and Petrie and MacAlister (1911), according to whom this reaction may serve to differentiate from Yersin's bacillus. Kurauchi (1931) insisted on the alkalization produced in four to seven days by Malassez and Vignal's bacillus in contrast with the acidification produced by Yersin's bacillus in 24 to 48 hours and the absence of modification by P. septica. Brigham and Rettger (1953) likewise see in it a distinguishing character from P. septica.

MacCoy, according to Moss and Battle (1941), considers this criterium as certain and gives as constant, after a slight initial acidification, a definite alkalization occurring at the end of three to four days to one week.

However, this alkalization is not admitted by all, and the absence of modification of litmus milk is mentioned by Klein (1899-1900), Lerche (1927), Truche and Bauche (1929), Barsini (1935), etc. Nikaronof (1927), Wu, Chung and Pollitzer (1936) even take away all value from this medium for differentiating Malassez and Vignal's bacillus from Yersin's bacillus due to the inconsistency or to the weakness of the alkalization by the first-mentioned bacillus and to the possibility of abnormal reactions with the second.

Devignat (1954) observed with five strains of Malassez and Vignal's bacillus "a very definite alkalization in a few days at 30° and a bluing after 24 hours of contact at 37° between a mixture of an equal part of litmus milk and of a thick suspension of non-

proliferative Malassez and Vignal's bacillus."

Of 103 strains that we examined, all produced alkalization in one week at 37°.

Litmus Whey: MacConkey (1908, then Otten (1926), suggested using this medium to distinguish the plague bacillus from Malassez and Vignal's bacillus. The slight acidification produced by the first-mentioned bacillus and the alkalization induced by the second were confirmed by Dujardin-Beaumetz (1934), G. Girard (1953), Knapp (1959).

An initial acidification phase usually precedes alkalization (MacConkey, 1908; Swellengrebel and Hoesen, 1915; Otten, 1926; Dumas, 1951).

Nevertheless, a number of authors did not observe any alkalization (Truche and Bauche, 1929) and others take all value away from this medium (Pirie, 1927; Miller and Gladky, 1927; Schütze, 1928).

98 strains of Malassez and Vignal's bacillus examined by us produced bluing of the medium in 24 hours at 37°.

g. Action on the red corpuscles.

Malassez and Vignal's bacillus is completely lacking in action on red corpuscles, according to almost all the writers on the subject (Roemisch, 1921; Baumann, 1927; Boquet, 1937; Terpilowska Rutkowska, 1938; Wilson and Miles, 1955; Bergey, 1957; Knapp, 1960; Percebois, 1961, etc.) or, according to some, it probably produces a slight hemolysis (Barsini, 1935) or a late hemolysis (Stephan, 1941) only exceptionally (Dumas, 1958). The absence of hemolysis followed by hemodigestion is reported by Henriksen and Jyssum (1961). A butyrous aspect of the colonies, although the gelose became clear and chestnut-colored, was mentioned by Mathey and Siddle (1954).

Certain writers have insisted on the lack of uniform sensitivity of the red corpuscles in various animal species. Thus Malassez and Vignal's bacillus probably hemolyzes red corpuscles in the horse, the rabbit and the guinea-pig, according to Korobkova (1940) [See Note] and in the sheep, according to Pomanskaya (1959).

([Note:] According to Communal (1945), Korobkova stated that Malassez and Vignal's bacillus did not hemolyze the red corpuscles in man and in the camel. We did not find this statement in Korobkova's article.)

According to Moss and Battle (1941), the human strain isolated by these writers, lacking in action on human red corpuscles, caused a beta type hemolysis on gelose with rabbit blood and the production of methemoglobin on gelose with meat extract and rabbit blood with the addition of 0.5 parts in 100 of dextrose.

The following are the conclusions of Harisijades (1953), after having studied the action of Malassez and Vignal's bacillus on the red corpuscles of various animal species at different temperatures and at various pH values: Malassez and Vignal's bacillus probably hemolyzes the red corpuscles of the rabbit, guinea-pig, horse and not of the sheep, ox, dog, rat, goat and chicken. This hemolytic activity depends both on the pH and the temperature. Thus the optimum pH is 8 for rabbit and guinea-pig blood and the optimum temperature is 37° for the hemolysis of the red corpuscles in the guinea-pig, 30° for the rabbit and 22° for the horse.

Devignat (1954) made the following observations with the R- and S-variants of three strains of Malassez and Vignal's bacillus: "There is no trace of hemolysis after 48 hours of incubation at 37°, but after 48 additional hours of cultivation at the ambient temperature, the R- and S-variants of two strains out of three are surrounded by a definite area of beta type hemolysis on sheep and rabbit blood but do not lyse guinea-pig blood. A third strain did not produce hemolysis after an additional observation for four days."

We examined the behavior, at 18°, 28° and 37° on gelose with horse blood at pH 7.4 and on gelose with rabbit blood at pH 7.8, of 120 strains of Malassez and Vignal's bacillus without observing the slightest trace of hemolysis after 4 days of incubation. Since Devignat had observed hemolysis only after 48 additional hours of observation at ordinary temperature, we re-examined our cultures under the same conditions. Only one strain that did not hemolyze horse or rabbit blood either at 18° or at 37° produced a slight hemolysis on gelose with horse blood after 48 hours of incubation at room temperature following an initial incubation for 48 hours at 37°.

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IV — VITALITY, PRESERVATION AND SENSITIVITY TO PHYSICAL AND CHEMICAL AGENTS AND TO BACTERIOPHAGE

1. Vitality in artificial culture mediums.

The long vitality of the germ in the various culture mediums has been observed for a long time. For Lucet (1898), one of the peculiarities of the germ is "the long time during which it preserves its vegetative power and its virulence in cultures. Cultivated on gelose, left in the incubator at 37° for three weeks, then preserved at laboratory temperature and in the light, it still provides very beautiful cultures when, after seven months, it is seeded again. In the same medium or in peptone bouillon it is still capable of growing after remaining in the incubator for three months at 37° and four months at 28°. A culture in bouillon, six months old, kept partly in the incubator at 28° and partly at laboratory temperature kills in three days with a 2 cc dose inoculated in the blood stream of an average sized rabbit."

Megnin and Mosny (1891) saw the vitality and the pathogenic power of cultures in gelatin preserved at 38° persist for four months and for 30 to 35 days with cultures in bouillon kept at the same temperature.

Truche and Bauche (1929), Dujardin-Beaumetz (1934), Lesbouyries (1941) insisted on the satisfactory vitality of the germ in bouillon and in gelose or in formulated serum under liquid petrolatum (Truche, 1938), but the best demonstrations of its good preservation were supplied by Polettini (1924) and Merlini (1938). Polettini, who transplanted in 1924 some cultures on gelose or in bouillon preserved in sealed tubes by Sacerdotti 22 years earlier, obtained, from the gelose tubes, cultures "that have preserved their virulence, although with a certain attenuation."

Merlini, who transplanted in 1938 four other cultures on gelose sealed by Sacerdotti at the same time as the preceding ones, that is 36 years earlier, obtained from one of them a culture whose virulence "although not completely gone, was, nevertheless, certainly attenuated in comparison with what Polettini had observed."

The preservation conditions and especially the temperature influence the vitality of the germ considerably. Although Lucet (1898), Megnin and Mosny (1891), etc. were able to distribute cultures after a more or less long period of preservation at 37°, this temperature is certainly one of the worst, as was first observed by Grancher and Ledoux-Lebard (1889) whose "cultures on agar 35 days old and preserved at 20° are fertile and virulent, whereas at 30° under the same age and seeding conditions they have lost their fertility and their virulence is doubtful, while at 37° they have lost fertility and virulence." Then it was observed by Gate and Bila (1928), according to whom "strains preserved at laboratory temperature stayed alive even after more than one month, whereas in the incubator at 37° the microbe no longer grows after two or three generations."

What we have said about the influence of the culture temperature on vitality, dissociation and virulence, applies likewise to the preservation of the strains.

Grancher and Ledoux-Lebard (1889) state, in addition, that "if the lack of virulence for the rabbit is considered as a sign of death,

it may be said that at 30° or 37° cultures on gelose live from four to five weeks. At 20° life lasts longer and we have not yet determined its limit."

Ramon (1914) believes that cultures in bouillon kept in the refrigerator preserve their virulence for several months, just as Basset (1946) does for whom preservation in deep gelose in the refrigerator keeps the vitality and virulence intact for at least eighteen months. We know that in fact, under these conditions, these periods of time have been exceeded by far.

According to K. F. Meyer (1958), cultures on a blood culture medium remain alive for years.

Personally, we consider as an excellent preservation process the use of formolated serum [See Note], covered with liquid petrolatum, preserved, after seeding, in the dark and at a constant temperature not exceeding 15° to 20°.

([Note :] To 500 ml of horse serum add one cc of commercial formol, mix; after a few moments of contact, add one cc of ammonia (at 22° Baume) to neutralize the formal-dehyde. Increase with two parts of distilled water and sterilize for fifteen minutes at 110°.

We were able to transplant some strains preserved under these conditions for 15 and 20 years, and, of several hundred strains, we never saw any lose their vitality or, appreciable, their virulence, taking into account individual variations in virulence from one strain to another. It appears to us that aging does not alter virulence very appreciably, in as much as we were able to establish it in our oldest strains. On the other hand, repeated transplantings are an obvious cause of weakening (Ravaglia, 1932; Dujardin-Beaumetz, 1934; Percebois, 1961, etc.). Basset (1946) considers that annual transplantings do not weaken virulence for at least five years, but he admits, nevertheless, a considerable attenuation toward the tenth year and advises, with Dujardin-Beaumetz, Lignieres, etc. transplanting some strains annually in guinea-pigs.

Lyophilized strains remain transplantable for years, according to K. F. Meyer (1958) and Knapp (1959). This process seems to us, however, to be definitely less good than preservation in Truche's medium or in gelose in the butt of a tube inoculated with a central injection. It appears, but this is still only an impression, that lyophilized strains undergo a rapid decrease in their sensitivity to the various bacteriophages used for an eventual lysotyping(P.Nicolle, 1962). On the whole, the conclusions of Heckly, Anderson and Rockenmacher (1958) on the lyophilization of Yersin's bacillus are valid for Malassez and Vignal's bacillus.

In practice, we use conjointly preservation in formolated serum and in gelose peptone water [See Note] distributed in tubes 8 mm in diameter and closed with rubber stoppers to avoid desiccation and seeded by central injection. The small amount of space taken up by this last-mentioned material facilitates its storage in the refrigerator at +4°C.

([Note:] Vaillant pepton, 10 g
Liebig extract, 5 g
NaCl, 5 g
Gelose, 10 g
Plain water, 1,000
Adjust at pH 7.4 - 7.5

Parallelly with these two methods, we also preserve our strains at -25°C, a temperature close to the -23°C recommended by Mead, Wessmann, Higuchi and Surgalla (1961) for Yersin's bacillus, but we have not yet had a sufficiently long period of time to evaluate this procedure.

2. Preservation in pathologic products.

The persistence of the germ in tissues kept at a low temperature was demonstrated by Moss and Battle (1941) who successfully inoculated guinea-pigs with macerations of pancreas and ganglions removed from a man who had died of septicemia caused by Malassez and Vignal's bacillus and preserved in the refrigerator at 40° F (or +4.4°C) for 16 days.

Knapp (1959) investigated systematically the duration of persistence of the germ in infected organs preserved at different temperatures, and concluded that the preservation period is at least three months at +2°C, from ten to twenty days at 22° C, from three to five days at 37° C and from one to two days at 45° C.

We also have observed the bacillus retain its vitality for two months at +4° C in organs (especially the spleen of guinea-pigs infected experimentally or in human meseteric ganglions).

The persistence of the germ in the stools of animals infected orally is irregular, but on the whole much shorter.

3. Preservation of the germ in nature.

The preservation of the germ in the outside environment has not been confirmed up the present time except by experiments that are at least very unreliable, since the determination was not always made by inoculation in the guinea-pig and never directly by culture. Under these conditions, the appearance of Malassez and Vignal's bacillus in guinea-pigs inoculated with earth (Grancher and Ledoux-Lebar, 1899), air collected on a wad of cotton (Chantemesse, 1887), milk (Parietti, 1890; Klein, 1900), water (Klein, 1889; Fraenkel, 1924), fodder (Lignières, 1898), liquid manure (Klein, 1889), does not in any way enable the statement to be made, far from it, that the germ was really present in the inoculated material.

Nevertheless, although it has not been demonstrated, the presence of the bacillus in the outside environment may be considered as certain (with reservations, however, with regard to the length of its persistence, its little resistance to light and desiccation), and it is spread by the stools (Paul and Weltmann, 1934) and by the urine (Lorey, 1911; Bryner, 1906; Dessy, 1925; Flamm and Kovak, 1958) of infected animals (Mollaret, 1960).

Morris (1958) demonstrated the persistence of the bacillus in samples of artificially contaminated earth or straw. We also observed that Malassez and Vignal's bacillus, like Yersin's bacillus, could remain alive for at least five months in sterilized earth, that has been sufficiently hydrated and preserved at room temperature.

The long persistence of Malassez and Vignal's bacillus in water must be admitted by virtue of the epidemiological consequences that it implies. According to Cecarelli (1950), the germ can resist autolysis for 95 days in distilled water at 37°. We confirmed this result.

4. Action of physical agents.

a. Resistance to heating.

The thermoresistance of Malassez and Vignal's bacillus has been various evaluated, according to the writer. Lucet (1898) has the honor of being the first to have insisted on its weak resistance to heat: "This microbe does not stand high temperatures well. Thus, it is killed, in a liquid culture medium, after staying six to seven minutes in a bain-marie heated to 55° and, in the dry state, fastened on silk threads, after having been subjected for two hours to a dry heat of 60°."

For Roemisch (1921), it resists for thirty minutes at 45° to 50° and, according to Zlatogoroff (1904), fifteen minutes at 57° and eight minutes at 60° in a liquid culture medium. Its resistance is greater on a solid culture medium on which it is probably not killed until after one hour and thirty minutes at 60°.

Most of the authors studied its resistance at 60° C, but they give results that are particularly discordant. Destruction at this temperature is probably accomplished in one minute , according to T'Hoen (1902), eight minutes for Zlatogorof (1904), ten minutes for Cecarelli (1950), Wilson and Miles (1955), Bergey (1957), thirty minutes for Saisawa (1913) and Dujardin-Beaumetz (1934), sixty minutes for Binato and Corrado (1960). Sachdeva, Kalra and Taneja (1956) succeeded in sterilizing cultures only after heating for two hours at 60°, and, according to K. F. Meyer (1958), saline suspensions are probably not uniformly destroyed after heating for three hours at 60°. According to Pfeiffer (1889), Delbanco (1896) and Poppe (1928), heating for one hour at 60° produces the loss of its virulence without altering its vitality, and heating for two hours is necessary to cause death at this temperature, results that agree with Knapp's (1959), according to whom culture suspensions in physiological water are not destroyed each time by heating for 180 minutes at 500-600 and may still yield a slow, discontinuous culture; heating for one hour at 550-600, on the other hand, destroys all the germs.

According to Messerschmidt and Keller (1914), their resistance is probably ten minutes at 66°, and one minute at 70°, according to Cecarelli (1950).

For Lignieres (1898), Malassez and Vignal's bacillus resists for ten minutes at 740-75° and dies at 840-85°. According to Sabin (1933) and Binato and Corrado (1960), it probably resists ten minutes at 600-800. This last temperature is probably endured for three minutes, according to Truche (1938). According to K. F. Meyer (1958) and according to Knapp (1959), the bacillus is killed in five to ten minutes at 700-800.

Tottire-Ippoliti (1916), who considers the germ to be very resistant, believes that damp heat (steam) kills it in thirty minutes and that it may resist forty-five minutes at 600-650, ten minutes at 80°, one to three minutes at 100° and is probably destroyed only after fifteen minutes at 80° and five minutes at 100°. The action of damp heat was also studied by Truche and Bauche (1929), according to whom "it resists damp heat for thirty to forty seconds at a temperature of 60°; at 80° it is killed in ten minutes, whereas it resists for three to five minutes at the same temperature" (sic).

At 100° heat kills it in ten minutes, according to De Blasi

(1908) and in thirty to forty seconds, according to [illegible] (1933).

None of these writers, unfortunately, specified the experiment conditions, especially the density of the cultures used or if heating was accomplished with or without stirring or shaking.

Knapp (1959) pointed out the part played by the experimental conditions and the lack of uniformity of resistance of the various strains. Of ten strains whose thermoresistance was studied partly on suspensions in milk, partly on cotton threads impregnated with dried culture, only two were killed after exposure to 60° for sixty minutes and all of them resisted one minute at 70°-74° and fifteen seconds at 85°. Only one to three strains were killed in one to two minutes at 85°.

On the whole, the thermoresistance of Malassez and Vignal's bacillus is weak and it must not be forgotten that the simple transplanting of cultures from 22° to 37° is always accompanied by a high mortality, as Preston and Maitland (1952) have observed.

b. Resistance to cold.

Pfeiffer (1889) reported that a period of seven hours at -9° or two hours at -16° did not alter either the vitality or the virulence of the germ. We learned, later, that low temperatures, far from being detrimental to its preservation, constitute, on the contrary, one of the best ways to ensure it. The experiments that we performed at -15°C and -25° C have so far given us excellent results.

c. Action of sunlight.

Malassez and Vignal's bacillus resits its action poorly (De Blasi, 1908; Saisawa, 1913; Poppe, 1928; Dujardin-Beaumetz, 1934; Lesbouyries, 1941). According to De Blasi, it is killed by an exposure of four to six hours to diffuse light. For Saisawa, a culture mounted on a slide is destroyed in thirty minutes by direct light; it resists eight hours in diffuse light, and twenty-four hours "in room light." Poppe confirmed the destruction of dried germs in thirty minutes of exposure to the sun.

d. Action of desiccation.

Dessication is also mortal (Delbanco, 1896; Saisawa, 1913; Poppe, 1928; Dujardin-Beaumetz, 1934). When the germs are placed in a desiccator, they are killed in five hours, according to Saisawa and forty-eight hours for Poppe and for Pfeiffer.

5. Resistance to chemical agents.

Sublimate: 1 part in 1,000 of sublimate kills Malassez and Vignal's bacillus "almost instantaneously", according to Dujardin-Beaumetz (1934), in one minute, according to Cecarelli (1930), in fifteen to thirty seconds, according to Tetire-Ippoliti (1916) and Lesbouyries (1941), in fifteen to thirty minutes, according to Truche (1938). 0.01 parts in 1,000 of sublimate kill it in two hours (K. F. Meyer, 1958).

According to Knapp (1959), concentrations of 1 part in 100,

0.1 part in 100, 0.01 part in 100 and 0.001 part in 100 kill it in the following times, respectively: immediately, thirty seconds, one-half to five minutes and 120 minutes.

Carbolic acid: According to Saisawa (1913), Malassez and Vignal's bacillus is killed in five minutes by phenol, 1 part in 100, and in less than two minutes by phenol in a concentration of 2 parts in 100. According to K. F. Meyer, the action of phenol, 1 part in 100, is probably slower, requiring five to thirty minutes. Phenol, 3 parts in 100, destroys the germ in two minutes, according to Cecarelli.

With a concentration of phenol 5 parts in 100, death probably does not occur for five to ten minutes, according to Truche (1938) and Lesbouyries (1941).

The figures given by Totire-Ippoliti (more than one hour's resistance in a 1 part in 100 solution and five to ten minutes in a 1 part in 500 solution), cannot be retained, due to the technique used by this writer.

According to Knapp, phenol in concentrations of 0.25 part in 100, 0.5 part in 100, 1 part in 100, 2 parts in 100 and 5 parts in 100 probably destroys the germ respectively in three to twenty-four hours, ninety to one hundred twenty minutes, five to thirty minutes, one-half to two minutes and, finally, in thirty seconds.

Sulfuric acid: The germ is killed in twenty minutes by sulfuric acid, 3 parts in 100 (Cecarelli).

Formaldehyde: Malassez and Vignal's bacillus is probably destroyed in one hour by formaldehyde, 4 parts in 1,000 (Truche and Bauche, 1928; Truche, 1938; Lesbouyries, 1941).

Individual differences in the sensitivity of the strains were pointed out by Hnatko and Rodin (1962), one of whose strains was killed by formaldehyde in a concentration of 1 part in 100 and another one resisted it.

According to Knapp, the germ is probably destroyed in thirty seconds by formaldehyde, 5 parts in 100, in five to ten minutes by formaldehyde, 0.5 part in 100 and 1 and 2 parts in 100, in ten to twenty minutes by formaldehyde, 0.25 part in 100 and in thirty minutes to one hour by formaldehyde, 0.1 part in 100.

Alcohol: Alcohol, 40 parts in 100, destroys the bacillus immediately, according to Saisawa, and alcohol, 60 parts in 100, kills it in one-half to five minutes, according to K.F. Meyer. According to Knapp, the destruction time is half a minute for alcohol in a concentration of 96 and 75 parts in 100, one-half to five minutes for alcohol, 65 parts in 100, and fifteen to thirty minutes for alcohol, 40 and 50 parts in 100.

Silver nitrate: Silver nitrate, 0.001 part in 100, kills the bacillus in two hours, according to K. F. Meyer, a time that is confirmed by Knapp who obtained the immediate death of the germ with a concentration of 1 part in 100, in one-half to five minutes with a concentration of 0.1 part in 100, in sixty to one hundred twenty minutes with a concentration of 0.01 part in 100 and in one hundred twenty minutes with a concentration of 0.001 parts in 100.

Iron sulfate and copper sulfate: Both destroy the germ in one hour in concentrations of 3 to 5 parts in 100 (Truche and Barthe; Lesbouyries; Truche).

Urotropin: Urotropin, 10 parts in 100, kills the germ in twelve hours (Cecarelli).

Soda: Soda, 4 parts in 100, destroys it in one hour (Cecarelli).

Dyes: According to Brigham and Rettger (1935), growth is inhibited by methylene blue, 1 part in 300,000, thionine and crystal violet, 1 part in 100,000, and brilliant green, 1 part in 1,000,000. The slight sensitivity of Yersin's bacillus and Malassez and Vignal's bacillus to gentian violet is the basis of Meyer and Batchelder's (1926) culture medium.

6. Cultures in "hostile" salt or bile mediums.

Sodium chloride: The addition of sodium chloride to a culture medium produces a slowing or a cessation of growth starting with a certain concentration along with the appearance of involution forms.

The concentration limit that is compatible with cultivation varies according to the authors: 2 to 3 parts in 100, according to Saisawa (1913), 6 to 10 parts in 100, according to Skorodumof and Somorovitch (1926), 8 parts in 100, according to Bonolnelli and Aradas (1932), 30 to 40 parts in 100, according to Percebois (1916), etc.

In fact, two points are to be considered: the nature of the medium and the incubation temperature. Indeed, it seems that the tolerance for sodium chloride, on the one hand, is slightly greater in liquid culture mediums than in solid mediums (thus, according to Smirnova, 1928, the inhibitive concentration is probably 3 parts in 100 in gelose and 4 parts in 100 in bouillon), and that, on the other hand, a given concentration is better tolerated at 22° than at 37°. Thus, according to Knapp (1959), a culture in salt bouillon, 3 or 4 parts in 100, is possible at 22°, whereas it is meager or absent at 37°.

In bouillon, the involution forms, a club or filament aspect or thick forms that are faintly and unevenly dyable, appear as soon as the sodium chloride concentration reaches 2 parts in 100. They are more frequent at 3 parts in 100 and dominant at 4 parts in 100 (Saisawa, 1913; Skorodumof and Somorovitch, 1926; P. Boquet, 1937; Topping and colleagues, 1938; Moss and Battle, 1941).

On salt gelose, 2 parts in 100, Saisawa (1913) obtained only a meager culture, while no culture appeared after three days on gelose, 3 parts in 100. On the same culture medium, Ravaglia (1932) obtained a good culture, rich in filamentous forms and emulsifying poorly in physiological water. Knapp (1959), when he cultivated twenty strains on salt gelose, 2 parts in 100, did not observe any modifications in the cultures or any involution forms at 22°, while at 37° fourteen of the strains displayed a definite slowing of their development with a preponderance long filamentous forms. These involution forms were more frequent on salt gelose, 3 parts in 100, and no strain developed on gelose, 4 parts in 100.

According to P. Boquet (1937), the filamentous elements de-

velop more readily at 37° on salt gelose, 3 parts in 100, whereas a higher concentration (5 parts in 100) probably favors the appearance of globose yeast-form elements.

A number of writers believed that they were able to differentiate Malassez and Vignal's bacillus and Yersin's bacillus according to their uneven capability for growing in hyerpsaline mediums (Saisawa, 1913; Skorodumof and Somorovitch, 1926; Dieudeonne and Otto, 1928; Haas, 1938; Pollitzer, 1954), but their opinions differ as to the results and as to the concentration required. Thus, a concentration of 6 to 10 parts in 100 probably inhibits the cultivation of Malassez and Vignal's bacillus, according to Skorodumof and Somorovitch, whereas, according to Boncinelli and Aradas (1933), gelose in a concentration of 8 parts in 100 probably permits the cultivation of Malassez and Vignal's bacillus and not of Yersin's bacillus. According to E. I. Smirnova (1928), neither of the two germs tolerates a higher concentration than 4 parts in 100 in bouillon and 3 parts in 100 in gelose, and this test would not enable any conclusion to be drawn.

The appearance of involution forms in one or the other of these germs on hypersaline gelose has also been invoked to differentiate them. According to Hankin and Leumann (1897), salt gelose, 3 parts in 100, produces the appearance of involution forms only in Yersin's bacillus. Galli-Valerio (1903), Wadsworth and colleagues (1947) confirmed this difference, whereas, for MacConkey (1908), the involution forms are probably not any more frequent in one of the germs than in the other.

These statements on the specificity of the involutional forms of Yersin's bacillus on salt gelose, 3 parts in 100, in comparison with the involutional forms of Malassez and Vignal's bacillus, were supported only insufficiently by Pollitzer (1954). Nevertheless, when it is cultivated on gelose, 3 parts in 100, Malassez and Vignal's bacillus probably presents a morphology that is different from the morphology of Yersins' bacillus under the same conditions. Thus, Topping and colleagues (1938), when they used this technique with their strains of Malassez and Vignal's bacillus of human origin, observed involution forms that looked "like ordinarily long, slender, slightly incurvate rods or average-sized rods gathered in bundles with an appearance resembling that of diphtheritic bacilli; alongside of these non-characteristic forms, round, voluminous forms, with a colored periphery and resembling the balloon-shaped elements produced by P. pestis in 24 hours on gelose with a 3 parts in 100 concentration of sodium chloride, were, on the other hand, more rare." According to Devignat (1954), the difference is uncertain: "P. pestis develops, on gelose with a concentration of 3 parts in 100 of sodium chloride, classic involution forms that have the general appearance of an irregular sphere bristling with roughness and pitted with vacuoles. Under the same conditions, P. pseudotuberculosis does not yield any forms that are more or less spherical but, rather, elongated elements, at times incurvate like a comma. Moreover, it seems, as Zlatogorof confirmed for the first time and as has been confirmed by other observers (Pollitzer), that the involutional forms of plague bacilli on salt gelose, 3 parts in 100, already appear in 24 hours, much more rapidly than with other species."

In a general way, these criteria rest only on shades of dif-

ference and neither the inhibition of the growth of either of these germs in hypersaline culture mediums, nor the morphological differences on these same mediums have the value that was initially assigned to them.

Action of bile: According to certain authors (Knapp, 1959), the cessation of multiplication or even the lysis of Malassez and Vignal's bacillus in culture mediums containing 25 to 50 parts in 100 of bile had probably been observed by Cernaianu (1928). In fact, Cernaianu, although he was speaking of "all the Pasteurella", seems indeed, to have had in mind only P. multocida.

P. Boquet (1937), Truche (1938) obtained no modification of culture in bouillon by adding 6 parts in 100 of ox bile, but they obtained a slowing of the growth with a concentration of 30 parts in 100.

According to Decouze (1934), the addition of bile to culture mediums inhibits cultivation without altering the vitality of the germ that remains transplantable, a result confirmed by Knapp (1959) who, although he did not observe any growth after four weeks of cultivation at 37° in mediums containing 50 parts in 100 of bile, did not observe any numerical reduction in the germs that had been seeded.

F. Kaufmann (1933), while studying comparatively the behavior of Malassez and Vignal's bacillus, of Yersin's bacillus and of P. septica in bile and salt culture mediums, did not observe any cultivation inhibition except in the last-mentioned of these germs. These results are close to the results of Wilson and Miles (1955) for whom Malassez and Vignal's bacillus and Yersin's bacillus give, in bile or salt mediums, liquid or solid, unsuitable for the growth of P. septica, a slight but definite culture that disappears in two or three days by autolysis.

Therefore, bile culture mediums are of no interest in differentiating Malassez and Vignal's bacillus from Yersin's bacillus (Pollitzer, 1954).

Action of sodium ricinoleate: Its action was studied by Reimann and Rose (1932) who observed a clarification of suspensions in physiological water up to a dilution of 1 part in 1,600. According to these writers, Spencer demonstrated that suspensions of Yersin's bacillus and of P. tularensis were completely clarified with 1/200 to 1/800 dilutions; suspensions of Malassez and Vignal's bacillus were partially clarified with a 1/25,600 dilution and completely clarified with a 1/800 dilution.

7. Sensitivity to bacteriophage.

Setting aside the theoretical problems raised by bacteriophages of Yersin's and Malassez and Vignal's bacilli, we shall take up here only their practical utilization.

According to Sugino (1932), Advier (1933), Bezsonova and colleagues (1938), Tumansky and Yastchuk (1938), plague bacteriophage is strictly specific to Yersin's bacillus. It probably does not produce any lysis of Malassez and Vignal's bacillus and it constitutes, therefore, a convenient and sure method of differentiation.

The first limitation on this specificity was made by P. C. Flu (1927) who demonstrated that plague bacteriophage was also capable of

lysing Escherichia coli. The opposite was demonstrated by Girard and Wollmann (1943) who established the sensitivity of Yersin's bacillus to certain dysenteric bacteriophages. Lazarus and Gunnison (1947) extended the lytic action of plague bacteriophage to the Salmonelleae. D'Herelle (1933), then Girard (1942 and 1943), observed that plague bacteriophage could display the same inhibitory power with regard to certain strains of Malassez and Vignal's bacillus as its specific bacteriophages. Devignat (1954) also obtained, by using Girard's plague bacteriophage, a lysis of Malassez and Vignal's bacillus, that at first was slow and incomplete, then rapid, after subjecting this germ to plague bacteriophage.

These results were confirmed by Lazarus and Gunnison (1947) who obtained the lysis of 19 to 27 strains of Malassez and Vignal's bacillus by a plague bacteriophage, followed by the lysis of all their strains after adaptation, and, the following year, by Gunnison and Lazarus (1948) who extended these results to 40 strains of Malassez and Vignal's bacillus.

Under these conditions, discrimination between Malassez and Vignal's bacillus and Yersin's bacillus by means of bacteriophage seemed to be impossible in practice, in spite of the reservations of P. C. Flu and H. Flu (1944-1945), but later studies by Gunnison (1950), Gunnison, Shevky, Zion and Abbott (1951) and of Gunnison, Larson and Lazarus (1951) showed that by working at 20° and by using a bacteriophage solution ten times stronger than the one that induces a confluent lysis ("critical testing dilution:), it was then possible to differentiate the two germs.

Cavanaugh and Quan (1953) proposed an easy diagnostic technique by using dried or lyophilized strips of filter paper preserved in sealed tubes under nitrogen after impregnation with plague bactiorophage. The application of one of these strips on a young culture of Yersin's bacillus on blood gelose incubated at 20°C produces, after 18 to 24 hours, a 1 mm lysis area, whereas, under the same conditions, no lysis is produced on cultures of Malassez and Vignal's bacillus.

Ul'Janova (1961) obtained lysis by means of specific bacteriophage of 37 strains of Malassez and Vignal's bacillus isolated by her from 1955 to 1959, while two were lysed by plague bacteriophage. In the same year, Plankina and Ogneva (1961) observed a similar sensitivity to plague bacteriophage in five strains of Malassez and Vignal's bacilli that came from marmots, while the bacteriophages isolated from four of these strains revealed themselves as active only on Malassez and Vignal's bacillus and on the only avirulent strains of Yersin's bacillus.

Knapp (1962), when he used one of G. Girard's bacteriophages that had never been in contact with Yersin's bacillus, concluded that it had a high specificity with regard to Malassez and Vignal's bacillus and he saw in it a supplementary diagnostic element for this bacillus, drawing up, however, limitations on its use in an area of plague endemy, limitations that are added to the ones formulated by G. Girard as early as 1942.

The various serotypes of Malassez and Vignal's bacillus seem to present appreciable differences in their sensitivity to bacteriophage. Knapp (1962) observed that the confluent lysis obtained on strains of types I, II, III and V is not found in strains of type IV that display only some isolated lysis areas. On the other hand, strains of types I, II, IV and V are little sensitive to bacteriophage

suspensions multiplied on type III strains. These observations are added to the ones made by Devignat in 1954 with the same bacteriophage: "The process of calculating the number of lysed areas brings to light a quantitative difference between the three varieties of P. pestis on the one hand and the species P. pseudotuberculosis on the other hand. Moreover, it results once more from the variable sensitivity to the same bacteriophage that the genus P. pseudotuberculosis is much less homogeneous than the genus P. pestis." We made the same observation in a liquid medium (see below).

From a practical point of view, the utilization of bacteriophage is a supplementary diagnostic element. If two tubes of peptone water or of bouillon are seeded simultaneously with a culture of Malassez and Vignal's bacillus and if one drop of bacteriophage suspension is added to only one of them, it is possible to see in this last tube, after the appearance of a cloudiness that is always more precocious than in the control tube, a rapid clarification that lasts a more or less long time. The time of appearance of the secondary cultures varies with the serological type of the strain used, whereas with type I, II, III and V strains the secondary cultures do not usually appear before the second, often the fifth or eighth day and even are completely lacking at times. The clarification observed in type IV cultures is often incomplete, and, especially, much shorter lasting. The secondary cultures appear commencing with the eighth hour.

Aside from this difference, the 617 strains in our collection all were revealed as sensitive to bacteriophage in a liquid culture medium, regardless of whether it was a question of Girard's "Yersin" phage, of Flu's "C" phage or of Girard's "PST" phage. These same bacteriophages produce a strictly analogous lysis on every strain of Yersin's bacillus that we used. This elementary technique, therefore, can be used only outside of every of plague endemy. Aside from this limitation, it constitutes a rapid and sure diagnostic method.

Although, also according to Girard (1953), the lysis of both germs, regardless of the bacteriophage used, has nothing specific about it, an indication may, nevertheless, be provided by the fact that the secondary culture always appears more rapidly in the tubes that have received heterologous bacteriophage.

The choice of a liquid culture medium for performing this test is not immaterial. As A. Chevalier has observed, secondary cultures always appear later in bouillon than in peptone water, with the difference between the two being able to be as great as several days. On the other hand, depending on whether the bacteriophage suspension comes from a filtrate of a culture in bouillon or in peptone water, the lysis obtained will likewise be of uneven duration. Secondary cultures always appear later when the bacteriophage comes from a culture in bouillon. Therefore, in practice it would be better to use this last-mentioned medium systematically, both for making the test and for multiplying bacteriophage, since the combination of both factors make the often incomplete and always transitory lysis more apparent in the case of type IV strains.

The influence of the medium used to multiply bacteriophage on the more or less long duration of the lysis that it produces must be distinguished from the influence of the medium on the power of this bacteriophage itself. A culture filtrate in bouillon in a 10^{-4} dilution will have the same activity as a culture filtrate in peptone water in a 10^{-6} dilution.

Finally, we can extract the following results from a study currently being conducted in collaboration with P. Nicolle and G. Brault:

A. Investigation of the lysogenesis of strains of Malassez and Vignal's bacillus:

When test strains endowed with a very broad sensitivity to numerous bacteriophages (S. typhi lysotype A, subtype Tananarive, S. paratyphi B lysotype 1, E. coli K 12 S, E. coli 36, E. coli Lisbonne, E. coli Bordet, Shigella paradysenteriae Y6 R, numerous strains of Salmonella pullorum et gallinarum, Malassez and Vignal's bacillus, etc.) are used, the results have always been negative, in agreement with the results that have already been published in this field.

B. Determination of the spectrum of sensitivity of Malassez and Vignal strains to bacteriophages:

a. Experiments on 25 March 1958: Twenty-four Coliphages from the collection in the Bacteriophage Service, tested on 15 strains of Malassez and Vignal's bacillus, allowed us to observe a great diversity of lytic reactions consisting of six different images:

Image 1: strains 4-I and 19-I [See Note]
Image 2: strain 21-I
Image 3: strains 35-I, 66-II, 82-II, 3-III, 78-V, 83-III and 91-V
Image 4: strain 46-III
Image 5: strain 75-V
Image 6: strains 32-I, 190, II and 210-I

([Note:] The Arabic numerals correspond to the numbers of the strains in our collection and the Roman numerals indicate their serological type.)

b. Second experiment (5 December 1961): Eighteen strains of Malassez and Vignal's bacillus were tested with 5 phages taken from drain-water and multiplied on the following Malassez and Vignal strains: 114-I, 233-I, 234-I, 276-I and 412-I.

There again a certain diversity in the reactions was observed, permitting the 18 strains to be divided into three lytic groups.

The first lytic group includes only type III strains: (strains 3-III, 149-III, 221-III, 452-III, 453-III, 454-III).

The second group includes 7 strains of type III (50-III, 83-III, 122-III, 123-III, 150-III, 162-III, 165-III), 1 strain of type IV (298-IV) and 1 strain of type V (52-V).

The third group comprises only type IV strains (51-IV, 95-IV, 151-IV).

In conclusion, by using 5 phages taken from drain-water, three lytic groups were obtained. The first and the third are antigenically homogeneous, since all the strains of one belong to type III and all the strains of the other to type IV. The second group, on the contrary, includes a majority of type III strains and a minority of strains of types IV and V.

As a result of these facts, type III can be subdivided into 2 lysotypes (lytic groups 1 and 2) and type IV also in 2 lysotypes (lytic groups 2 and 3).

c. Third experiment (11 July 1962): 13 phages were used, 8 of which came from drain-water and 4 were preparations adapted from phage 1 of the complementary lysotyping of lysotype A of S. typhi (Nicolle, Pavlatou and Diverneau's lethod, 1953, competed by Nicolle, Diverneau and Brault, 1958 (P. Nicolle, G. Diverneau and J. Brault, Bull. Res. Council Israel, 1958, 7E, 89-100).

These phages were tested on 27 strains of Malassez and Vignal's bacillus, 5 of which were type I, 6 type II, 8 type III, 4 type IV, 1 type V and 3 of an undetermined type.

The results of this experiment demonstrated that by means of these 13 phages the strains of Malassez and Vignal's bacillus could be grouped in six lysotype groups according to the sensitivity spectrum of the strains.

The first group (sensitive to all the phages, except to phages 3, 5 and 7) includes 3 strains all belonging to type III, strains 3-III, 122-III and 149-III).

The second group is sensitive to all the phages except 4 (1, 3, 5 and 7). It comprises 5 strains of type III, 2 strains of type IV, 1 strain of type II and 1 strain of an undetermined type.

The third group is sensitive to all the phages except 5 (1, 2, 3, 5 and 7). It includes 5 strains of type I (strains 500-I, 502-I, 503-I, 504-I, 512-I), 1 strain of type II (strain 400-II), 2 strains of type IV (strains 95-IV and 298-IV) and 2 strains of an undetermined type (strains 489 and 505). This group, therefore, does not contain any strain of type III or of type V.

The fourth group is sensitive to 7 phages, which are phages 4, 8, 9, 10, 11, 12, 13. It includes only one single strain of type II (strain 398).

The fifth group is sensitive only to 2 phages (4 and 10). It includes two strains (479-II and 480-II), both belonging to type II.

Finally, the sixth group is resistant to all the phages except one (12). Likewise, it includes one single strain (103-II) that belongs to type II.

The results of this last experiment confirm and expand the results of the first two. They indicate clearly:

a. That the strains of Malassez and Vignal's bacillus show rather varied spectrums of sensitivity to a set of bacteriophages.

b. That some of these spectrums are homogeneous from the antigenic point of view; that is to say that they contain only one single antigenic type (first group: type III; fifth group: type II). This homogeneity suggests an interesting relationship between the antigenic structure of these strains and their lysotypic image.

c. That certain antigenic types may be subdivided into several lytic groups. Thus type III strains can produce the reactions of lytic groups 1 and 2; type II strains were distributed in 5 lytic groups (lytic groups 2, 3, 4, 5, and 6); type IV strains can be subdivided into 2 lytic groups (2 and 3).

On the other hand, all the type I strains, 5 in all, were classified in lytic group 3 that contains, nevertheless, a small number of strains belonging to other antigenic types (1 strain of type II, 2 strains of type IV and 2 strains of an undetermined type).

d. Some antigenic types are not represented in certain lytic groups, although these groups were not revealed as homogeneous from

the antigenic point of view. Thus, no type I strain appears in lytic groups 1 and 2 and no type III strain appears in lytic group 3.

In view of such results, one has, therefore, the right to think that it is possible to lysotype Malassez and Vignal's bacillus by means of phages taken from drain-water and phages from various other sources. Of course the proof of the epidemiological value of such a lysotyping remains to be established.

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V — BIOCHEMICAL IDENTITY OF THE VARIOUS STRAINS AND THE CASE OF SEROTYPE IV

Lerche (1927) and Schütze (1928) demonstrated that all the strains studied by them, although coming from different animal species, formed a remarkably homogeneous group in their biochemical behavior. Knapp (1960) did not find any cultural, biochemical or serologic differences between strains of human origin and strains of animal origin either.

The systematic study of the 617 strains in our possession did not enable us, any more so than the above-mentioned authors, to discover the slightest difference, regardless of the animal source, the geographic origin or the age of the strains.

On the other hand, if the behavior of the various serotypes of Malassez and Vignal's bacillus that are known at present is considered, certain peculiarities can be found among the type IV strains, to the exclusion of any other type.

The acidification of sodium citrate is usually faster (2 to 4 days, generally, at 18° to 20°) with this type of strains than with the others that require, on the average, two to five weeks.

Only type IV strains ferment sodium malonate at 18° and at 20°. Although this character is not constant since it is lacking in one of our six strains of this type, we found it, nevertheless, in this group alone.

Sensitivity to specific bacteriophage is definitely less in type IV strains. Inhibition, when it is studied in plain bouillon, is always less definite and more transitory and the secondary cultures much more precocious. Knapp (1962) also observed that the confluent lysis obtained in types I, II, III and V strains was missing from the four type IV strains examined by him and which displayed only areas of isolated lysis.

The sensitivity to certain antibiotics, especially colistatin, of type IV strains is also different from the sensitivity of other types of strains (Courtieu and colleagues, 1961).

The absence [See Note 1] or at least only occasionally their small pathogenic power (Thal, 1953, 1955, 1956, 1962) also contributes to their individualization [See Note 2].

([Note 1:] This character is not constant, however. Bouton and Hall (1961) isolated, in an acute case of mesenteric adenitis in a child, one strain of type IV that was as virulent as the other types of strains. This strain was the only one of this type that did not ferment malonate.)

([Note 2:] This avirulence for mammals peculiar to type IV strains is no longer found, for example, in the louse. Krynski and Beola (1962) observed that type III, IV and V strains were equally virulent in a louse that had been infected anally, in contrast with strains of type II and especially of type I.)

Although the number of type IV strains in our possession (six) is too small to authorize a formal conclusion, it is none the less true that these peculiarities, in spite of the exceptions pointed out in the notes to the preceding paragraph, provide this type with a certain originality within the group of Malassez and Vignal's bacillus that is, moreover, so homogeneous.

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VI — RELATIONS WITH YERSIN'S BACILLUS AND TAXONOMIC PROBLEMS

Two questions must be discussed here: on the one hand, the relations of Malassez and Vignal's bacillus with Yersin's bacillus, on the other hand, the relations with the other species that at present are still classified in the genus Pasteurella, and the position that Malassez and Vignal's bacillus must occupy definitively in systematics.

a. Relations with Yersin's bacillus.

This problem is summed up in two questions: what are, from a practical point of view, the factors that enable the two germs to be differentiated, and what is, from the dogmatic point of view, their exact degree of relationship? Although, from the viewpoint of the cultural and biochemical criteria, it is now easy to answer the first question, the second one is an infinitely more delicate matter.

From a practical point of view, two tests suffice to to differentiate Malassez and Vignal's bacillus from Yersin's bacillus to a certainty: investigation of mobility and determination of urease. Although, among the innumerable criteria that have been proposed during the last sixty years, the unevenness in the rapidity of cultivation and the disparity in the nutritive requirements of the two germs, the differences in pH observed during their development or the differences in behavior noted on certain culture mediums (potato, litmus milk, deaerated mediums, Leifson's medium, etc.) or in the presence of certain carbohydrate substances (rhamnose and glycerol most of all, but also melibiose, etc.), the differences in sensitivity to the bacteriophage detected under special, precise circumstances, etc., constitute, indeed, valuable arguments, none of them, nevertheless, is as valuable as the two tests mentioned above: mobility below 30° and aptitude for decomposing urea, that are as consistently present in Malassez and Vignal's bacillus as they are consistently absent from Yersin's bacillus, are, alone, sufficient for their differentiation. If the question of the priority to be assigned to one of these two tests should be raised, we would assign it to the determination of urease. Although, in fact, certain strains of Malassez and Vignal's bacillus are probably capable of losing their mobility definitively, either, according to Favorisova (1938), due to mere aging (we have not, however, observed it ourselves in strains preserved without transplanting for more than twenty years), or, according to Brunet (1952), due to action of bacteriophage, the use of this latter technique has never enabled us to obtain mutants incapable of decomposing urea.

In fact, the real problem does not seem to us to be the differentiation of the two germs, but rather the determination of their exact degree of relationship. Therefore, we shall emphasize here primarily some parallel factors:

We shall do no more than mention, without underestimating their importance but because they lie outside the framework of this study, the analogies that exist in the antigenic and physio-pathologic sphere: the existence of close antigenic relations that have as their consequences the possibility of crossed vaccinations,

the analogy of behavior in Xenopsylla cheopis (Blanc and Baltazard, 1944), and, finally, the analogy in the pathogenic modalities. In connection with the last point of view , it must be emphasized that, although there are differences, they are exclusively quantitative and are due only to differences of degree in virulence, while the ganglionic tropism is identical in the bubonic forms of the plague and the mesenteric forms of the infection due to Malassez and Vignal's bacillus, the pulmonary or septicemic manifestations of both diseases are identical and the toxins of the two germs, so similar in their nature (Girard, 1950), have the same neurotropism. Finally, the histological substrate of the two infections also is completely superposable: "plague is a pseudotuberculosis", according to Dujardin-Beaumetz.

Therefore, the only difference seems to be, finally, merely a question of degree in virulence, however, this calls for the following remarks: septicemias caused by Malassez and Vignal's bacillus in man were, before antibiotic therapy, just as mortal as the plague septicemias themselves. Experimentally, the same animal species (dog and cat) prove to be immune to both germs, and, especially, the supreme differentiation criterium that was constituted for a long time by the susceptibility of the white rat to Yersin's bacillus and its immunity to Malassez and Vignal's bacillus, must be reconsidered since the isolation, by G. V. Juscenko (1957-1959), of strains of Malassez and Vignal's bacillus that are highly pathogenic for the rat and always more pathogenic for the white mouse than for the guinea-pig. Finally, this virulence criterium is particularly weakened if one takes into account the existence of spontaneously avirulent strains of Yersin's bacillus or, as Girard (1947) has demonstrated, of strains that lose part or even all of their virulence after contact with bacteriophage.

The susceptibility of both germs to the same antibiotics and especially to the same bacteriophages also constitutes a remarkable similarity factor at the same time as it demonstrates in the best way the fragility of the biochemical differentiation criteria to the extent that Korobkova (1937) was able, by means of this technique, to make Yersin's bacillus acquire the ability to ferment rhamnose and to the extent that Brunet (1952), on the contrary, was able to make Malassez and Vignal's bacillus lose its locomotor apparatus.

If one is satisfied with the only two differentiation criteria that we have definitively retained, mobility and decomposition of urea, the following remarks must be made: in so far as the mobility of Malassez and Vignal's bacillus is concerned, on the one hand its disappearance under the special conditions that we have pointed out weakens its dogmatic importance considerably, on the other hand its strict subordination to temperature conditions must be reconciled with the analogous influence exercised by temperature on a number of characters of Yersin's bacillus, for example on its nutritive requirements (Hils and Spurr, 1952). In so far as the decomposition of urea is concerned, we believe that this character cannot constitute, by itself, a sufficient distinction at the "genus" level or even at the "species" level and it must be retained only at the "variety" level, like the fermentation of glycerol, the reduction of nitrates or the production of nitrous acid.

Under these conditions, we believe that Malassez and Vignal's bacillus should be compared with Yersin's bacillus on the same "variety"

level as the varieties that Devignat (1951) individualized under the terms "orientalis", "antiqua" and "mediaevalis" in this last mentioned species, since this comparison has, like the subdivisions proposed by Devignat, in addition to a biochemical basis, a geographical basis constituted by the fact that Malassez and Vignal's bacillus has never been isolated in the tropical zone, particularly in the southern Sahara, the Belgian Congo, Madagascar or Viet-Nam, as Girard (1960) emphasized.

We consider, finally, therefore, that both species, Yersin's bacillus and Malassez and Vignal's bacillus, that are, at present, still separate and classified one under the genus Pasteurella (Trevisan, 1887), the other under the genus Cillopasteurella (Prevot, 1948), ought to be brought together, as shown in Table I, below, in the same genus Yersinia, proposed by Van Loghem (1944-1945; 1946).

TABLE I

genus	species	variety	urease	glycerin	nitrates
Yersinia	Yersin's bacillus	orientalis	0	0	+
		antiqua	0	+	+
		mediaevalis	0	+	0
	Malassez and Vignal's bacillus		+	+	+

Is it possible to develop the comparison further? Already, in 1928, Zlatogoref and Mogilevskaya considered Malassez and Vignal's bacillus to be an R-variant of Yersin's bacillus, and Harvey, in 1933, as a plague bacillus in symbiosis with a bacteriophage. Subsequently, Devignat saw in these two germs the mutants of a same original species, and, in order to explain the disappearance of the medieval plague from Europe, published, in 1951, the hypothesis of a transformation of the mediaevalis variety of Yersin's bacillus into Malassez and Vignal's bacillus, a hypothesis that is added to Levine's (1913) and Boncinelli and Aradas's (1933), who had admitted, from a theoretical point of view, the possibility of transition from one to the other germ.

Have these hypotheses begun to be confirmed? In 1912, Rowland reported that by cultivating the plague bacillus in a solution of its own nucleoprotein he had succeeded in transforming it into Malassez and Vignal's bacillus, but the discrimination criteria admitted by this author, based only on degrees of virulence, prevent us from subscribing to his conclusions. The same is true for the variants of Malassez and Vignal's bacillus that Zlatogoref and Mogilevskaya obtained in 1928 and Pokrovskaya in 1934.

In 1936, Bessonova, Lenskaya, Moloditsova and Mossolova reported five cases of spontaneous "transmutation" of Yersin's bacillus into Malassez and Vignal's bacillus that had occurred between 1929 and 1936 in 214 strains preserved in a collection.

Tumansky reported, two years later, a case of transformation, under the influence of a plague bacteriophage, of Yersin's bacillus into a germ "that resembled very closely the pseudotuberculosis

bacilli", a result that was close to Korobkava's who, in 1937, produced, by also subjecting Yersin's bacillus to the action of a plague bacteriophage, a series of modifications among which were the loss of virulence and the acidification of rhamnose culture mediums.

Faddeeva stated, in 1939, the progression of these transformations as follows: The virulent strains of Yersin's bacillus are transformed into avirulent strains of the same germ which in turn are transformed into R-form avirulent strains of Malassez and Vignal's bacillus, and then into S-form virulent strains of this last-mentioned bacillus.

Finally, although all these observations report transitions of Yersin's bacillus to Malassez and Vignal's bacillus, an opposite phenomenon was reported by Blanc and colleagues (1944; 1962) as a result of experiments on the behavior of Malassez and Vignal's bacillus in Xenopsylla cheopis.

A particularly pertinent discussion of these observations will be found in Pollitzer (1954) and, especially, in Girard (1947). Indeed, none of these observations has been able to be repeated every time (Pirie, 1929) or even to provide a demonstration, really above all criticism, of the reality of mutations of one species into the other (therefore Blanc and we have limited ourselves to writing in connection with one of these "transformations" that "everything occurred as if ..."), but it is none the less true that they form a group of facts that are, to say the least, disturbing and that plead also in favor of a more than close relationship between the two germs.

b. Relations with the other species of the genus Pasteurella and taxonomic problems.

An examination of Table II, below, shows sufficiently that between Yersin's and Malassez and Vignal's bacilli, on the one hand, and Pasteurella septica or multocida, on the other, there are strictly no characters that justify keeping these three germs classified any longer in the genus Pasteurella (Trevisan, 1887), and no one could have expressed better than Girard (1942) the need for separating the first two germs from Pasteurella in the strict sense: "the development of the plague and pseudotuberculosis bacilli in yeast water and in potato water, the absence of flocculation of a serum antiplague filtrate of Pasteurella culture, the total absence of lysis of the Pasteurella genus by an antiplague bacteriophage whose polyvalency and activity are very pronounced, add still more to the bundle of arguments invoked for separating Yersin's and Malassez and Vignal's bacilli from the Pasteurella genus. In fact, what do they have left in common? Bipolar staining, due to which they were formerly integrated into the same genus. Let us recognize that at present this character has nothing specific about it."

The production of indole and the fermentation of sucrose by P. septica, and the non-fermentation of glycerol, of rhamnose and of esculin, the absence of antigenic relations (except for the lipopolysaccharide isolated from P. multocida type I by Bain and Knox in 1961), the difference in behavior in the hematophagous insects, the presence of a tetrathionate-reductase and the absence of beta galactosidase confirm again, as if it were necessary, the contrast between P. septica on the one hand and the Malassez and Vignal's and Yersin's bacilli on the other.

Finally, the presence in comparison with these three germs of a species as different in its morphology and in its cultivation requirements as the agent of tularemia, Pasteurella tularensis in the present state of nomenclature, also calls for a division of the genus Pasteurella whose nomenclature and taxonomy have been shown by Talbot and Sneath (1960) to be incorrect.

We have already outlined (see page 2 , above) the regrettable practical consequences of the present classification and the ambiguity created by the term Pasteurella. To the extent that nomenclature has the objective "of avoiding or rejecting the use of terms and names that may cause errors or ambiguity and become, consequently, a source of confusion" (Haudroy, 1949), we believe that the term Pasteurella and the genus that it designates should be reserved for the single species P. septica. We subscribe then fully to the proposal to group Yersin's and Malassez and Vignal's bacilli in the new genus Yersinia (Van Loghem, 1944), as we do to Dorofeef's (1947) proposal, taken up by Olsufiev and colleagues (1959) and by Philip and Owen (1961), to put the agent of tularemia in the new genus Francisella (Dorofeef, 1947).

TABLE II

DIFFERENTIAL CHARACTERS OF MALASSEZ AND VIGNAL'S BACILLUS, OF YERSIN'S BACILLUS AND OF PASTEURELLA SEPTICA

		Malassez and Vignal's bacillus	Yersin's bacillus	Pasteurella septica
Mobility at 20°		+	0	0
Urease		+	0	0
Indole		0	0	+
cultivation on	potato	+	0	0
	yeast water	+	+	0
	hydrolysate of gelatin or Berkmann's medium	+	+	0
	MacConkey's medium	+	+	0
	Morris's medium	+	+	0
Sucrose		0	0	+
Rhamnose		+	0+	0
Glycerin		+	0+	0
Esculin		+	+	0
Methyl red		+	+	0
Tetrathionate reductase		0	0	+
Beta galactosidase		+	+	0
Antigenic relations		+	+	0
Bacteriophagic relations		+	+	0
Persistance in *Xenopsylla cheopis*		+	+	0

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